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RCS MEDDH - 288 (RI)

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RESEARCH IN BIOLOGICAL AND MEDICAL SCIENCES

Including

BIOCHEMISTRY, COMMUNICABLE DISEASES AND IMMUNOLOGY,
INTERNAL MEDICINE, NUCLEAR MEDICINE, PHYSIOLOGY,
PSYCHIATRY, SURGERY, AND VETERINARY MEDICINE.

ANNUAL PROGRESS REPORT

1 July 1964 - 30 June 1965

VOLUME 3

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WALTER REED ARMY INSTITUTE OF RESEARCH

WALTER REED ARMY MEDICAL CENTER

WASHINGTON, D.C. 20012

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RCS MEDDH-288 (R1)

RESEARCH IN BIOLOGICAL AND MEDICAL SCIENCES, INCLUDING
BIOCHEMISTRY, COMMUNICABLE DISEASES AND IMMUNOLOGY,
INTERNAL MEDICINE, NUCLEAR MEDICINE, PHYSIOLOGY,
PSYCHIATRY, SURGERY, AND VETERINARY MEDICINE

(Projects, tasks, and work units
are listed in Table of Contents)

Annual Progress Report
1 July 1964 - 30 June 1965

Volume 3

Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington, D. C. 20012

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SUMMARY

The various subjects covered in this report are listed in the Table of Contents. Abstracts of the individual investigations are included on the DD Form 1498 introducing each work unit report, and names of investigators are given at beginning of each report.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care as established by the National Society for Medical Research."

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PROJECT 3A025601A824
IONIZING RADIATION INJURY, PREVENTION AND TREATMENT

Task 01
Ionizing Radiation Injury, Prevention and Treatment

| RESEARCH AND TECHNOLOGY RESUME | | | | 1. GOVT ACCESSION | 2. AGENCY ACCESSION | 3. REPORT CONTROL NUMBER |
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| 12. SCIENTIFIC OR TECH AREA | | | 13. START DATE | 14. CRIT. COMPL. DATE | 15. FUNDING AGENCY | |
| 014000 Radio and radiation chemistry | | | 05 59 | NA | OTHER DA | |
| 16. PROCUR. METHOD | 17. CONTRACT GRANT | 18. DATE | 19. RESOURCES EST. | 20. PROFESSIONAL MAN YEARS | 21. FUNDS (in thousands) | |
| C. IN-HOUSE | NA | | PRIOR FY 65 | 14 | 283 | |
| | | | CURRENT FY 66 | 14 | 312 | |
| 22. GOVT LAB INSTALLATION ACTIVITY | | | 23. PERFORMING ORGANIZATION | | | |
| NAME Headquarters | | | NAME Walter Reed Army Institute of Research | | | |
| ADDRESS U.S. Army Medical Res & Dev Command | | | ADDRESS Washington, D. C. 20012 | | | |
| 24. INDIV | | | 25. INVESTIGATORS | | | |
| Goldstein, Col. J.D. | | | PRINCIPAL Jacobus, Dr. D.P. | | | |
| 202-0X 65957 | | | ASSOCIATE Sweeney, Dr. T.R. | | | |
| 26. TECHNOLOGY UTILIZATION | | | 27. COORDINATION | | | |
| Drug development; chemical industry | | | NA | | | |
| 28. KEYWORDS | | | | | | |
| Activity; chemical; compound; dose; drugs; protection; radiation injury; structure | | | | | | |
| 29. (U) Tech Objective - The objective of this research is to develop a militarily useful pill to protect personnel against the lethal effects of ionizing radiation. In addition to a strictly tactical military use an efficient antiradiation compound would be useful to the Army from the clinical standpoint. | | | | | | |
| 30. (U) Approach - Approach to the objectives is through accepted drug development protocols. Synthesis and testing of potential agents is being carried out. Test results are analyzed for structure activity relationships and fed back into the synthesis program. Promising compounds are carried forward to testing in large animals and the pharmacology of these compounds investigated. In addition chronic toxicity studies, dose reduction factor studies and drug antagonism studies are being carried on. The development of efficient methods of handling chemical and biological information which can be applied to the program are being developed. | | | | | | |
| 31. (U) Progress (Jul 64 - Jun 65) - Progress continues to be made in the development of useful agents. The position rather than the presence of a ring in a candidate agent has emerged as an important factor in the lipid soluble class of compounds. It is to be looked at in the water soluble class. Absorption after oral administration continues to be a problem in the lipid soluble class but surprisingly good absorption and protection have now been obtained with certain compounds in the water soluble class. Excellent protective indexes have been obtained with certain compounds. New ideas in related chemical structures are being investigated. For technical reports, see Walter Reed Army Institute of Research Annual Progress Report, 1 July 1964 - 30 June 1965. | | | | | | |
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| 35. MISSION OBJECTIVE | | 36. PARTICIPATION | | | | |
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DD FORM 1498

1 Aug 64

(Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 813 & 815C WHICH ARE OBSOLETE.

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ANNUAL PROGRESS REPORT

Project No. 3A025601A824
Task No. 01
Work Unit No. 055

Title: Ionizing Radiation Injury,
Prevention and Treatment -
Chemical Protection Against
Irradiation

Description: The development of an antiradiation agent for man.

Progress:

I. General

The Antiradiation Drug Development Program continued to make satisfactory progress over the last year. There are several specific criteria for the estimation of the degree of progress in the last 12 months. The index of safety (originally referred to as an index of interest) has risen from approximately 15 to 35. Considering that we started with an index of 1.5, this quantitative measure of safety indicates a considerable development over the last five years and a very appreciable development in the last year.

The index of the potency of the compounds under development is called the dose reduction factor. At the time this program started, the dose reduction factor was in the order of 1.6. We now have single agents with dose reduction factors slightly over 2, and combinations of agents with dose reduction factors approaching 3. While this is small, five years ago we felt that it would not be possible to increase the potency of these agents. Dose reduction factors vary appreciably if the strain or age of animal are changed. We have mice with dose reduction factors over 3, but these animals are not used as our regular screen. Last year, of course, we reported dose reduction factors in excess of 3 for dogs.

The third criteria by which one might judge the progress of the program is the increasing sophistication of the chemical structures under development. Mercaptoethylamine is fundamentally a very simple molecule. Analogs are now available of such appreciable size, complexity, and different properties that solid hope for the development of an effective agent can be entertained.

Lastly, sub-acute toxicity studies in monkeys have been carried out without demonstrating toxicity, thereby paving the way for the administration of some of the agents to human volunteers.

The overall level of effort in the program remains approximately the same. There has been a mild acceleration in the rate of screening of off-the-shelf compounds and a mild reduction in chemical synthesis rate.

The chemicals under development in the Antiradiation Drug Development Program can be classified into two broad groups: The lipid soluble and water soluble materials. The lipid soluble compounds have as their advantages the following facts: 1. they offer the highest index of interest of any agents in the program; 2. they are effective when administered in small doses, thereby permitting consideration of reasonable pill sizes; 3. they appear to be longer lasting than the water soluble materials. They of course differ appreciably in their physical characteristics from the water soluble derivatives with which the program started. These lipid soluble derivatives have gained in sophistication in the last two years, and now incorporate a fair number of rings and a few heterocycles which have been effective in protecting against radiation injury. These heterocycles are the only compounds in the radiation program in which rings have been successfully used. The lipid soluble derivatives have as their disadvantages the following facts: 1. they were not absorbed from the gastrointestinal tract; 2. that they tend to produce cardiac toxicity when administered in toxic concentrations; and 3. that they, of course, produce emesis. Until this past year, they had the additional serious disadvantage that they did not combine with any of the water soluble class of antiradiation agents. This lack of combination ability was such a serious consideration as to raise doubts about the mechanism of action of the lipid soluble derivatives in comparison to the mechanism of action in the water soluble compounds. DURING THIS LAST YEAR, it was discovered that the water soluble thiophosphate derivatives would combine with the lipid soluble compounds in order to offer an increased protection against radiation injury. In the combination studies, the highest protection factors are those obtained when the lipid and the water soluble derivatives are used in combination. One effort of the past year, which is expected to extend over the next two years, is the fusion of the lipid and the water soluble compounds. Accordingly, some polar groups are being introduced into the lipid soluble derivatives in order to have gained enhanced water solubility and thus obtain the advantages of the water soluble groups while retaining those advantages accruing to the lipid soluble groups. During the past year no simple combinations of

lipid and water soluble features were attempted, such as was done two years ago. Instead, effort was placed on raising the index of safety and attempting to develop more sophisticated rings down the lipid chains. These efforts are expected to continue. During the last year another generalization also became apparent. The lipid soluble derivatives are most effective if the sulfur function is either a mercaptan or a thiosulfate. Lipid soluble derivatives in which the sulfur function is a thiophosphate are of much reduced activity if they are active at all. Disulfides of this class have some action. Thiolsulfonates appear to be comparable in potency to the corresponding Bunte salts.

When discussing the disadvantages associated with the lipid soluble derivatives, one can only express complete frustration with the inability of these compounds to be absorbed across the gastrointestinal tract. Four or five candidates in this family have been found to be absorbed poorly and some beginning structural rules may be developing which will permit the design of agents which will be more regularly and more effectively absorbed. In general, however, gastrointestinal absorption of this series of materials is poor. We feel at the present time, that we should continue to push this series of agents in spite of their poor gastrointestinal absorption because of the advantage outlined above. But, we are, however, setting up special studies in order to see if the structural features associated with gastrointestinal absorption can be developed.

The cardiotoxicity information has been based in the past primarily upon gross electrocardiographic observations in the anesthetized dog and upon semi-quantitative studies done by Captain Demaree and Dr. Aldo Corbascio of the University of California on the reduction of the contractile force of the isolated guinea pig atrium. By virtue of these tests (which consume only small amounts of material) agents have been developed which appear to be markedly less cardiotoxic.

Emesis with the lipid soluble agents appears to be less pronounced than emesis with the water soluble materials. Sophisticated studies with the lipid soluble agents have not been carried out because of the difficulty of obtaining these materials in solution at an interesting pH. It is, however, our impression that emesis in the lipid soluble agents tends to become less of a problem as the agents develop an increasingly higher index of safety, so that we hope this problem will gradually become less and less severe.

The water soluble chemicals have as their prime advantage the following facts: 1. they appear to protect in the same manner as the classical agents; 2. they have the highest dose reduction factor (potency); 3. they lack cardiotoxicity; 4. they are well-absorbed by mouth. Since the protection by these structures appears to be the same in its effects, it must be considered that this class of agents has as one of its advantages the studies done on the basic mechanism of related compounds by radiobiologists, world wide. The dose reduction factors, i.e. the degree of potency obtained with one group of these water soluble compounds, namely the thiophosphates, appears to be the highest of any of the single antiradiation agents. The thiophosphate derivatives combine well with the lipid soluble Bunte salts to offer an enhanced protection. Very closely related water soluble substances, such as mercaptans, corresponding to the thiophosphate antagonize the lipid soluble Bunte derivatives, thus demonstrating in a dramatic manner the unusual features to be associated with a change in the covering function on the sulfur part of the MEA derivative.

The water soluble derivatives have as their disadvantages the following facts: 1. they must be administered in large doses; 2. they appear to be of relatively short duration of action; 3. they produce emesis. This large amount of material, which must be injected for protection, is the basis for stating that the water soluble derivatives are not quite as far along as the lipid soluble derivatives in terms of being ready for clinical administration at levels which will protect against ionizing radiation injury. These compounds appear to be of relatively short duration. They are also capable of producing emesis. In the case of the water soluble derivatives, the thiophosphates appear to be the most active. Analogous Bunte salts result in a diminution in protective action. Amides and other unusual polar groups have been developed in the past year with a reduced emphasis upon hydroxyl groups. As a result, the potential class of interesting agents has been broadened. The water soluble groups have also been administered in the diet over a considerable period of time. Lathyrism has been noted with some of the water soluble derivatives. This lathyrism is most pronounced in young mice who receive the agent in an abnormally high percentage in the diet. Mice receiving the material at a level of approximately 3 g/kg of body weight have developed lathyrism. No lathyrogenic changes have been noted in monkeys to which the material has been fed for very considerable periods of time. For example, MEA has been administered over one year to monkeys in a gradually increasing level up to 100 mg/kg per day in the diet. The agents which produce lathyrism are also effective in the in vitro test system for rheumatoid

factor as developed by Dr. Israeli Jaffe. Whether there is any fundamental correlation between these two phenomena remains, of course, to be seen. The water soluble derivatives are also effective in protecting against nitrogen mustard injury in contradistinction to the failure of lipid soluble derivatives to protect against HN_2 . Since protection against nitrogen mustard is going to be one of the bases for administering large quantities of these agents to patients having terminal malignancies, this feature of antagonism of the action of radiomimetic chemicals is important.

During the past year there has been a gradual increase in the amount of material required from the prep laboratories. When the prep labs were originally instituted, a fair number of chemicals were requested in 100 gram amounts. The second year in the existence of the prep labs, requests were in the kilogram range. We now have a number of requests which have been in the 5 kilogram range. These increasing amounts of material are necessary in support of the subacute Food and Drug-like studies which have become increasingly more numerous. The prep labs have faced real chemical problems associated with these scale-up procedures which have required very often that they run a series of preliminary runs and tests in order to find an optimal way to make the candidate material. One of the prep labs has inadvertently synthesized a very interesting chemical of unknown structure related to one of the promising antiradiation compounds. We do not anticipate that any of these chemicals would be difficult to obtain on a full-scale production basis, but the difficulties of making the initial scale-up lot should not be underestimated.

The Pharmacology Department, in addition to providing its usual series of excellent reports on the pharmacodynamics associated with these agents, has been very active in the development of small test systems. Two years ago these test systems were limited to isolated organs. Lately, the development of the anesthetized mouse for cardiovascular pharmacologic effects makes possible the development of cardiovascular data with a very small amount of material. The lipid soluble agents also appear to be interesting on the basis of their interference with catechol amines. The Pharmacology Department is planning to extend their studies to central nervous system pharmacology.

A number of special tests relating to drug tolerance have been carried out over the past year. 1. It was discovered in the monkeys that the administration of increasingly large quantities of mercapto-ethylamine would result in an increased tolerance. Monkeys on a flexible dose level actually developed an increased tolerance for the

material so that they eventually would consume without any untoward effects 100 mg/kg per day. These studies are reported in detail in a report by the Woodard Research Corporation. 2. Dr. I. Jaffe found in his studies that penicillamine would produce leukopenia when given to people. A special experiment was carried out primarily at the Woodard Research Corporation in order to determine if a similar pattern of penicillamine administration would also produce leukopenia in rabbits in the same manner that long alkylisothiourea derivatives produce leukopenia. Penicillamine did not produce leukopenia in rabbits when given according to these schedules. 3. The tolerance of the dog liver for perfusion with the water soluble alcohol mercaptoethylamine derivatives was also carried out. These drugs have as their prime manifestation of toxicity acute liver pathology believed to be due to spasm of the hepatic vein with subsequent edema, hepatic engorgement, and portal hypertension. These studies have been reported in the past. Liver perfusion studies in patients with terminal malignancies will permit us to obtain in man, selective protection of the normal liver while providing some benefit in the treatment of the metastatic liver nodules. Therefore, with the development of this procedure, we expect to obtain in man tolerance studies for massive doses of anti-radiation compounds. In view of the sensitivity of the liver to these agents, we feel that this perfusion study will provide us with the opportunity to observe the liver while the drug is being administered, and have the critical organ under direct control. These perfusion studies, therefore, enjoy a very high priority. Some of the new agents in the alcohol series are being tried. At the present time, the only compounds considered appropriate for the antagonism of HN_2 are the free mercaptans.

Data processing in the antiradiation program continues to be a problem which is not easy to solve. It is, however, proceeding very much better than before, primarily as of Captain George Orthey and Captain David Davidson's endeavor in this area. All the mouse radiation data is processed up to date and error free. Programs exist and are functioning for the distribution of acknowledgement of compounds, results of recent radiation tests, correlation by source and the printing of summary data.

A. Discussion of Recent Chemical Data

The information which is to be presented in the following chemical section is a direct continuation of the information presented for the last two years. The structures that will be discussed should be

considered as being in many cases part of the series, the antecedents of which were reported last year. There will not be any attempt to repeat last year's data so that of necessity, there will be need when following a series of compounds to refer to the previous annual progress report. In this discussion, we will attempt to bridge the gap between the previous work and the work herein reported in appreciable detail.

The same format for reporting previous work will be maintained in this current report. The reader should be reminded that the index, herein called the index of interest (or the index of safety), increases as the compound is tested at a low level with respect to the estimated LD_{50} . Another way of stating this phenomenon is to say that the index becomes higher as the compound has a greater spread in activity. This index is to be distinguished from the dose reduction factor. We have compounds with a high index that have only an average dose reduction factor and vice versa.

The compounds reported in Figure 1 represent derivatives from the long alkyl lipid soluble antiradiation agents typified by 1607. WR 1607 for purposes of comparison has a protective index of 6. This year consider that unless the index is 10 or more, the compound is not interesting at all. The first compound reported in Figure 1, WR 3341, is tested quite close to the approximate LD_{50} . It produces 40% survival. The resulting index of 2.2 is no interest. In general, it can be seen that all the other variations reported in Figure 1 are essentially uninteresting. WR 3298 is a nine carbon derivative which is tested quite far away from the approximate LD_{50} , i.e. at the half dose offers good protection, but at the quarter dose no protection. The nine carbon compound has a higher index of protection. This compound has one aspect to it which is interesting: namely, it is absorbed to an appreciable extent by mouth and when absorbed does offer some protection. In general, the overall doses for this structure are so large that this is one of the less interesting Bunte salts except for this feature of oral absorption. WR 3561, the cyclohexylbutyl derivative, has the advantage that only a small amount of material is required for effective action. However, there is very little action at the half dose and the index is not interesting. WR 3613, the cyclobutylpentyl derivative has good protective action at 12 1/2 mg/kg. This cyclopentyl derivative is also absorbed by mouth but to a much lesser degree than the cyclopentyl butyl. When absorbed by mouth it does produce radioprotection.

Figure 2 shows three compounds for purposes that are new but illustrate certain lessons which have been learned in the past. 3823 is a straight alkyl bunte derivative shown here for purposes of comparison with 3358. The intermediate short chain compound is not active. It presumably falls in the range of inactive Bunte salts represented by the normal pentylaminoethylthiolsulfate. 3358 on the other hand is a 10 carbon branched alkyl bunte derivative. This compound, originally made at WRAIR, has good activity down to 1/8 of the maximum tolerated dose. We had originally difficulty with the branching of long alkyl chains in the sense that the branched compounds lost their activity. This compound is indicative of enhanced activity as a result of branching. It should be pointed out that our most active compounds in this series are now those having branches. The index of 20 is to be compared with the index of 6 for the normal decyl analog. As with the normal decyl analog, this compound is not absorbed by mouth. The short chain thiol phosphate 3679 has minimal activity demonstrating that the conversion of the Bunte salt to the thiol phosphate does not confer activity upon the short alkyl derivatives.

Figure 3 shows a set of compounds in which the insulating chain is 4 normal carbons from the nitrogen of the aminobunte derivative. These compounds are an attempt to exploit the Phenbutyl and cyclohexylbutyl derivatives reported last year to have good antiradiation action, and also reported last year to have relatively little peripheral vasoconstrictive action and relatively little cardiotoxic action as measured by the tests on the guinea pig atrium, in comparison to other derivatives in this same family. Also included in this series of compounds is an attempt to introduce some polarity either through phenyl or methoxy groups so as to favor gastrointestinal absorption and to edge the lipid soluble derivatives towards the direction of being somewhat more water soluble. All of these compounds tend to have relatively large doses of material required for protection in comparison to the compounds having no polar groups. However, the increase in doses are perhaps not tremendously large. 3299, the phenol derivative, made as the most polar one in the series is not interesting even when tested at a level of 150 milligrams per Kg. 3050, the paramethoxy derivative is a highly interesting compound in that the maximum test dose is 30 milligrams. This compound is also a depressant in the same sense that 1607 is also a depressant and does not produce convulsions in mice. The compound is absorbed to some extent if it is administered by mouth prior to exposure to radiation. In general, as will be seen from later work, the paramethoxy substitution does confirm some gastrointestinal absorption on the compound in question. The ortho derivative

3614 is also interesting in that it too is a depressant with activity that holds up well at the quarter and eighth doses. But, the dimethoxy derivative, 3338, is not interesting at all. WR 3614 is also a depressant and, therefore, has this as an additional desirable feature. We anticipate that proper handling as methoxy groups or rather simple alkyl ether groups on the phenyl nucleus may well tend to confer some anti-emetic activity upon these structures. But, both 3050 and 3614 do not contain sufficient alkyl substitution to do anything significant with respect to antiemetic activity if structural analogs with other series can be relied upon.

Figure 4 shows the effects of varying the ring substituents after 4 insulating methylene groups were interposed between the ring and the amine. In view of the excellent activity of the phenbutyl Bunte salt, the effect of adding two methyl groups was considered. WR-3340 and 3347 are essentially comparable in terms of the test dose and the percent survival. Neither of them have indexes of interest nor are either of them absorbed by mouth. Surprisingly WR-3342 is absorbed by mouth to some minimal extent showing the advantage of a paraethyl substituent. This is the first compound in which this effect of the paraethyl substituent has been demonstrated. WR 3564, 3339 and 3558 are aliphatic analogs of the above aryl substituents. The latter compounds are less active than the present cyclo butyl compound. In view of the low activity of WR-3558, it was not tested by mouth.

Figure 5 is a limited exploration of heterocyclic Bunte salt derivatives with 3818 included for purposes of comparison. WR 3818 is one of the few examples of compounds made this year in which the nitrogen of the mercaptoethylamine moiety is close to the ring. As found before, we have further shown that the benzyl type nitrogen in the mercaptoethylamine moiety destroys activity. WR 3085 and 3609 exploit the insulating function of the normal butyl group. WR 3085 is comparable to the phenbutyl Bunte salt analog. As can be seen from the indexes, the compound is interesting. It differs somewhat from the phenbutyl compound in the amount of material needed for the test dose. It is not absorbed by mouth. WR 3609, tryptophane analog is also tolerated at relatively high levels. It also demonstrates insufficient activity to merit further consideration.

Figure 6 is a further exploration of the 4-methylene insulating function. This time the attempt was to increase the complexity of the ring in order to see if a lower dose or a more prolonged effect could be achieved. Unfortunately with these compounds, there was not sufficient activity to merit continuation of this particular approach.

Figure 7 is an attempt to explore variation on the insulating chain with one compound, WR 3552, again included for purposes of comparison since it was made this year. The dramatic compound in figure 7 is WR 3562 in which the butyl chain is varied so that the ring substituent is in the 2 position. This compound is therefore a structural analog of the cyclo-hexylbutyl derivative. The LD₅₀ is relatively high at 300 mg/kg but the compound demonstrates protective action all the way down to low levels. It therefore has a very high index. In addition, this compound is a depressant so that it has significant possibilities for advance testing. The phenyl n-hexyl compound was found not to have sufficient activity to merit further investigation. The corresponding phenoxy derivative 3821 has no activity. WR 3567 is not sufficiently active to merit further attention, but the concept of an aliphatic ether having interesting properties appears to be suggested by the fact that there is some action there.

The study of the influence of an ether function is continued in Figures 8 and 9. The phenoxy derivative is not active. It is therefore strictly comparable to 3821 in figure 7. However, the o-tolyl analog 3087 has significant action. The bases for these differences are not understood. WR 3087 is not absorbed by mouth. WR 3335 has relatively good activity at a high level which unfortunately does not hold up. This lack of activity is also noted with 3565 which is strictly analogous to 3342, but this time lacking in activity. WR 3343 also has some action but not enough to merit further investigation. WR 3336 maintains the tolyl feature associated with the good activity of 3087. It is effective at a lower dose, but it is not effective by mouth. The meta-chloro compound 3819 does have a significant spread of activity. The same spread can be seen for the dichloro derivative 3122 except that there is appreciably more toxicity when the compound is administered at levels above 25 mg. WR 3610 is not interesting, showing there are limits to how far the chain can be extended.

Figure 10 is an exploration of the advantages and disadvantages of 3 insulating carbons between the nitrogen and the ring. Specifically, 3301 examines the possibility of whether one methylene can be replaced by an oxygen since it is isosteric. A complex function such as 3304 if it were active would have the advantage of offering considerable variations so that the program might be able to get into new kinds of structures. Unfortunately, it does not seem to be effective. WR 3300 examines the advantage of an orthomethoxy derivative with the propyl chain and it does not appear to be interesting.

Figure 11 is a further exploration of variations on the insulating function, this time preserving the phenyl substituents for the most part

so as to have a constant basis for comparison. All the variations tried, as can be seen, resulted in compounds which are essentially uninteresting.

The conclusion drawn from figures 4 thru 11 is that the branched butyl chain in which the ring is on the 2 position is definitely of interest and perhaps other branches need to be explored. Secondly, the replacing of one or more methylenes by an ether or thioether function does not result in compounds that are active, neither does the introduction of an oxygen into the side chain. In view of the significant interest with the branched butyl group and the overall importance of the butyl insulation, this substituent will be examined in the forthcoming year.

Figure 12 consists of a series of compounds which are acylated in order to attempt to break the sevidiron nature of the Bunte salts or the corresponding alkyl mercaptans. As can be seen from a quick glance at the protection data, the compounds are essentially inactive against radiation injury. This inactivity may result by virtue of the fact that the mouse excretes the compound so rapidly that there is not a sufficient build-up of material for protection in spite of the very large amount administered. Judging by the slight difference in protection between 3423 and 3605 the diformyl derivative has slightly more activity suggesting that mice are capable of deformylation but not of de-acetylation. The other compounds are not active in spite of an attempt to vary the chain links of the substituent groups and to include examples of materials which are effective if the nitrogen is not acylated.

Figure 13 consists of dialkylated versions of compounds which have also been made as a mono-amine derivative. These compounds developed mainly as byproducts during the synthesis of the more desirable compounds. However, as with other tertiary amines in the past, these compounds are not active.

Figure 14 represents an attempt to exploit the acids and amides reported in the annual progress report last year. These esters or acid Bunte derivatives lack activity. They do, however, constitute a fundamentally new series of structural considerations. WR 3553 is the methyl-ester of a corresponding interesting compound if the sulfur is present as the mercaptan. The hope was with the historification of the compound the increased lipid solubility would make the Bunte salt an active compound. WR 3554 follows the same line of reasoning in addition to invoking the branch chain arrangements now found to be effective. WR 3680 is an extension of the same reasoning with the expectation that the increased numbers of ester groups would confer lower toxicity.

Such expectation was realized although the compound is still ineffective. It is our feeling that one of them should perhaps be tried as the corresponding thiophosphate. Moving the acid group down the chain or increasing the complexity also appears to eliminate radiation protection. No further modifications are expected to be done along the line of 3597 or 3133.

On Figure 15, WR 3683 and 3682 are extensions of the above minor reasoning based on oil versus water solubility. The increasing molecular weight of the side chain was designed to see whether activity could be obtained in these compounds if the right quantity side chains were used. This reasoning is exactly parallel to the reasoning by which the original compounds of 1607 were thought to have activity. 3608 is the Russian version of an active compound. As can be seen, the compound has very little activity, thus following our previous experience in difficulty in exploiting combinations of compounds using the so-called Russian substitution (the methyl group on the carbon carrying the thiol).

On Figure 16 there are a series of compounds which can be considered to have as the common feature a carbon or carbocyclic substitution on the carbon carrying the amine function. WR 3542 is the cyclopentane version of MEA in which both the nitrogen and the mercaptan are on the same side of the ring. This compound, therefore, tends to keep the mercaptan and the amine relatively close to each other. It should be compared to MEA in which case it can be seen to be slightly less toxic and at the half dose to hold up perhaps a little better than MEA. This compound which is difficult to make is, therefore, somewhat superior to MEA. We have not made many variations on this structure using this basic ring in place of MEA because of the difficulty in the synthesis of the material. When the final mercapto-amine drug is developed, then it might be desirable to try the corresponding cyclopentane version in order to see if an additional modicum of protective action can be obtained. In exploring the possibility of hindering the carbon carrying the amino group WR 3349, the cyclopentane version, is not active if the sulfur is as a mercaptan, but the thiophosphate does confer a reduction in toxicity and an increase in protective action. The decyl Bunte analog of 1607 does not appear to be active in spite of the fact that it is administered at a much higher millimolar level than 1607. This line of substitution is not expected to be pursued in the future.

Stanley Brois had a number of phenylaziridines available which permitted the easy development of the material shown on Figure 17.

They offered the possibility that the appropriate optical isomers would confer protection. This series, in general, confirms the experience found with mercaptans; namely, that if the phenyl group is on the carbon carrying the sulfur function, the series is usually inactive.

Figure 18 represents an extension of the hydroxyl series begun almost two years ago. As can be seen from a superficial inspection of these compounds, the molecular weight of the side chain has been increased somewhat with the expectation that there would be some increased lipid solubility. There is also a consistent combination of hydroxyl with amine functions in the side chain with the hope that the advantages associated with the amino alkyl amino thiophosphates could be combined with some of the advantages associated with the simple hydroxyl series. On Figure 18 these compounds are all mercaptans since the corresponding thiophosphates in this series have been difficult to make. These mercaptans came about essentially as byproducts from the corresponding thiophosphate synthesis. The two compounds combining hydroxyl amine functions, 3100 and 3094 still have the large dose associated with the hydroxyl series, but do not appear to do as well as either the simple amines or the simple hydroxyl derivatives. WR 3345 and 3356 were attempts to gain a lipid soluble group; namely, an ether linkage in this structural series. When hydroxyls are still present as with 3345 the compound has some protective action, but when the simple alkyl ether is used there is no protective action. WR 3345 might be interesting as the thiophosphate. WR 3297 and 3120 represent hydroxyl series in which the lipid-like nature of the compound has been increased in one case by substituting dimethyl functions, in the other case by adding a fluoroethyl group. Both of these compounds are administered at dose levels which are relatively low for the hydroxyl series, but unfortunately neither of them appear to have sufficient activity against radiation injury to merit further development. The suggestive evidence from this series is that the intimate combination of alkyl functions, or fluoro alkyl functions and hydroxyl groups is not a promising way to merge the lipid and water soluble classes of compounds.

Figure 19 demonstrates further work in the series combining hydroxyl with Bunte functions. This series, in general, appears to have the same properties as hydroxyl groups combined with mercaptan functions, mainly the dose levels are high and the indexes are 4 or less. This series is notable for the fact that there is protective activity at all because we had previously felt that the combination of the short chain hydroxyl function with the Bunte salt would not lead to any protective action. While this series of structures serves to increase the potential use of the Bunte salt with hydroxyl groups, the success of this

class is obviously dependent upon an appropriate manipulation of the nitrogen side chain so as to obtain compounds which are effective at lower test dose levels.

Figure 20 contains the disulfide versions of a fair number of hydroxyl series reported so far. These compounds, in general, were available as a result of attempts to synthesize the corresponding thiophosphate compounds. While they were obtained as essentially by-products in the synthesis, they were intentionally made because they were easy and because we had previously felt that covering a mercaptan function with a disulfide group was appropriate in the case of the hydroxyl series. As can be seen from a general inspection, these compounds reflect the activity seen in the corresponding mercaptans in addition to being administered at approximately the same level as the corresponding mercaptans. These compounds have the advantage of being more stable than mercaptans. In terms of radioprotection, however, they do not appear to have any advantage over other versions of the same series. The disulfide compounds might well have been expected to offer longer protection in terms of duration of action. While these duration of action studies have not been carried out, the suggestive evidence based on the lack of protection with low levels of 3568 suggest that the protective action would not last for an exceptionally long period of time.

Figure 21 is a collection of compounds in which the side chains have weak nitrogen functions insulated by an appropriate side chain from the corresponding sulfur functions. Some of these compounds are bis-structures, with the expectation that such a modification might increase the potential activity of the series. 3600 compared to 3598 can be seen to be effective at much lower levels; however, the protective action does not appear to be as pronounced. 3350 also has less action than the corresponding one-armed derivative 3599. In general, this series is not as promising as the corresponding mercaptan series with the exception of 3598 which probably merits structural modification as the corresponding thiophosphate in view of the comparable activity obtained between 3598 and the corresponding aminopropylaminoethylmercaptan. These weak nitrogen functions should have much less tendency to release histamine than the corresponding strong amine functions.

The synthesis of the thiophosphate derivatives has proceeded slowly this year due to technical difficulties in spite of the series receiving high priority, therefore, only a limited number of new structures have been added. Figure 22 shows a series of three structures which were

selected from the series of bis-mercaptans as being potentially interesting to make as thiophosphates. These compounds were designed to cover the interesting range in which m equals 3 or 4 and then determine if lack of interest would be demonstrated in the thiophosphate series comparable to the mercaptan series when m was 5 or 6. The results bear out that this parallelism does, in fact, exist. 3694 is an interesting compound in that it holds up at relatively low dose levels, lower levels in fact than the corresponding non-bis structure again demonstrating the utility associated with this class of compounds. The corresponding one-arm structure is 2721 shown in Figure 23. In view of the interest in the aminobutylaminoethylthiophosphate, it will pay to make the corresponding bis structure during the forthcoming year.

Figure 23 demonstrates further work with the corresponding aminopropylaminoethyl thiophosphate and the corresponding non-amino acridine. During the year a large quantity of 2721 was obtained, thereby permitting the conduct of combination studies. Figure 23 is a duration of effect study. 2721 is given at 600 mg/kg at increasing time intervals of 60, 180 and 300 minutes prior to radiation. The survivals are written on the right. Non-amino acridine, 2921, is given at 25 mg/kg. 60, 180 and 300 minutes prior to radiation with the corresponding survivals also written on the right. The combination of the two materials at the same time intervals is then reported. It can be seen at 60 and 180 minutes prior to irradiation the survival obtained is that obtained through the use of the thiophosphate alone, but at 300 minutes the combination of the two materials offers increased protection. At the present time we are not certain how fundamentally useful this combination of materials will be.

Figure 24 is a continuation of the same work shown in Figure 23 in which additional studies on non-aminoacridine are reported. The attempt here was to build up the compound through repetitive administration in order to see whether protection would result. There was increased toxicity associated with multiple injections of the material with some variable results. In general, however, it can be seen that there was essentially very little build-up in protection associated with 2921 even when there was appreciable toxicity associated with the administration of the structures. It is our feeling, therefore, that 2921 does not contain any appreciable radioprotective action in its own right.

Figure 25 represents a continuation of the attempt to develop new structural features associated with the basic mercaptoethylamine nucleus.

These compounds are characterized by having an amidine function associated with a Bunte group. These compounds continue to demonstrate activity in comparison to the cyclohexyl Bunte amidine diversion reported several years ago. They also have the advantage of being effective by mouth at relatively low levels and appear to hold up well when administered 60 minutes prior to exposure to irradiation injury. In spite of the small effort in amidine series on the basis of these results, we feel it desirable to continue with these structures for at least another year.

Figure 26 represents a series of new nitrogen functions which were made at WRAIR. These compounds are fundamentally different from previous antiradiation agents inasmuch as the nitrogen function is weak. This group of materials, therefore, represents a class of new antiradiation agents which has not yet been explored, but in view of the different characteristics of this nitrogen they do need exploration. These compounds still have the disadvantage that they require large amounts for protection. However, the aromatic nature of the side chain does permit the possibility of substitutions. This series, therefore, represents an important development which will take a couple of years to exploit, but which has the possibility of offering appreciably different final products. Fortunately, this series has the advantage of being relatively easy to synthesize in comparison to some of the other classes of antiradiation agents.

Figure 27 represents an attempt to extend the weak nitrogen functions associated with new covering groups. These structures represent rather drastic structural modifications from those compounds previously tried and found to be effective. As can be seen from an inspection of the survival data, none of these compounds was associated with protective action, although one of the desired objectives, mainly the obtaining of compounds which would be administered at low levels, was reached with a number of the structures. This series further tends to amplify that the conversion of the MEA nitrogen into an amide function or three carbons between the nitrogen and the sulfur is not associated with protective action.

Figure 28 also represents a further attempt to break out of the MEA class. 3556 probably does not have a strong enough nitrogen to permit transguanilation. It also corresponds to the corresponding butylisothiurea which is reduced in activity. 3318 is quite surprising in comparison to 3557 in that it is appreciably more toxic. Our hope here was that the ethoxy group would be metabolized off allowing the compounds to have protective action without the corres-

ponding increased basicity associated with the guanadine derivatives. Unfortunately, they do not appear to be active against radiation injury.

Figure 29 represents the beginning development of a series of new covering functions. 3538 is really a model compound made in order to explore the chemical feasibility of this series. 3596 is a corresponding n-acetyl version of this compound using this new covering function. Unfortunately, with this modification it does not appear to be effective although it does have the advantage of being used in relatively low doses in comparison to the n-acetyl MEA. 3569 represents a new sulfur function in which there are three sulfurs in a row. There does appear to be some protective action associated with this compound. It is certainly effective at levels much lower than the corresponding n-acetyl. It has more protective action than the n-acetyldisulfide; therefore, this compound may be interesting as a potential covering function. 3584 had a third covering function which does not appear to be protective, at least if the MEA is acetylated. All of these new covering functions were tried with the n-acetyl version of MEA for structural considerations, but additional modifications were planned in view of the overall importance to this program of the development of new covering functions.

Figure 30 contains one compound which is an extension of covering functions studies. Compound 3629 is a variation on the thiosulfonate of Lamar Field which proved to be exceedingly useful as an antiradiation agent. This compound, unfortunately, does not appear to have sufficient protective action to merit further development. 3353 and 3351 represent the thio and Bunte variations of alkyl sulfone derivatives. In spite of the appreciably large difference in the tested doses, neither of these compounds appears to have significant protective action.

Figure 31 represents a series of compounds which were obtained without cost from Bill Foye of the Massachusetts College of Pharmacy. These compounds have the advantage over the corresponding ligands in that they would be administered at levels of approximately 1/10th the dose level. In contradistinction to the other chelates previously reported, these compounds do not have protective action.

Figure 32 represents a collection of structures which indicate an attempt to vary at various degrees structural modifications designed to broaden the characteristics of the aminothiols antiradiation compounds. 3547 probably has anticolon ester rays action. It does not appear to be effective against radiation injury. 3136 lacks protective action. This

is probably not surprising in view of the tertiary nature of the amine and the thio ether substitutions. However, it does not appear to be very toxic. 3687 represents a combination of the good covering function discovered by Lamar Field two years ago along with an aromatic version of the MEA in order to see if this covering function would make possible the development of a series in which the MEA could be converted into an aromatic molecule. This particular attempt was unsuccessful. We do feel, however, there is some justification for exploring these new covering functions with aromatic conversions of the fundamentally aliphatic MEA. 3310 represents an attempt to make the thiophosphate analog of the animopropylaminoethylthiophosphate acridine derivative. This amine function in non-amino acridine is a strong base in that the parent compound has a PKB of 3×10 to the minus fifth. The compound should, therefore, have an activity comparable to the corresponding amide. Such a structural variation would be very desirable if it could be developed, in view of the known tendency of the acridine series to localize in DNA and to stay in the body for long periods of time. This compound which has been extensively studied in our laboratories under multiple regiments does not appear to be effective against radiation injury. 3543 represents an attempt at a new covering function. Unfortunately, it is not effective against radiation injury. The compound is interesting as an ancillary consideration because it is appreciably more toxic than the corresponding potassiumthiosianate; whether it has a use in the treatment of hypertensive disease remains undetermined. An additional quantity of 462 was obtained in the process of the synthesis of 3687. This compound does not appear to have protective action.

In Figure 33 there is a collection of additional structures in which the MEA moiety is hidden in a ring structure. The lead for this series of compounds was developed last year as a result of the success with 2950 synthesized by Dr. Herbrandson at Rensselaer Polytechnic Institute of Technology. Additional information on 2950 is presented in Figure 34. As can be seen, this compound has the advantage of providing activity at relatively low test dose levels in addition to being effective by mouth. Larger quantities of this material are not yet available, but the activity in this series prompted the attempted several structural variations in order to obtain action. 3541 in Figure 33 is the closest analog to 2950. This compound is not effective against radiation injury. 3357 is a very near relative to 3541. It was hoped that 3692 and 3352 would be relatively labile and so release in vivo mercaptoethylamine. Judging by the protective action, this hope does not appear to be substantiated. Other relatively wide structural variations on this lead are also reported in 33, but, unfortunately,

found not to have activity. In Figure 34 WR 3325 can be seen to be very close to 3357. The conclusion of the results obtained with 3325, 3357 and 3541 is that substitution in the ring so as to make a thiazoline is not a promising substitution. 3306 is a similar maneuver.

Additional competence in the protective action on 2950 has been developed during the last year. This compound is effective in protecting mice when administered orally. It, therefore, represents one of the first long chain derivatives which offers action when administered orally. It also has the advantage that it is administered at relatively low levels although not as low a level as usual with the ordinary 1607 class of antiradiation agents. However, the data is suggestive that the structural modification associated with the rise in index and the reduction in the amount of material required for the other derivatives will be effective here. Those variations are, therefore, planned for the forthcoming year as well as studies in higher animals using 2950.

B. Chemical Synthesis Program

1. Contract

The chemical synthesis program for FY 65 has operated on a \$750,000 budget. The status of the current contracts and the changes in the status of contracts that have occurred during FY 65 is shown in Table I. Table II shows a breakdown of the types of active contracts for FY 65. This includes one non-synthetic contract. The remainder are of the synthetic type. A breakdown of the number of compounds and the approximate cost per compound for the various types of contracts for FY 65 is given in Table III.

The chemical synthesis program has continued to move along two general lines: namely, the lipid soluble and the water soluble agents. This is an arbitrary division and there are many compounds that cannot be unqualifiedly categorized. However, this division helps in correlating the numerous compounds submitted. In general, the lipid soluble agents are more effective as Bunte salts, whereas the water soluble agents are more effective as phosphorothioates or mercaptans.

It was noted in last year's report that problems had arisen in the purification of the polyhydroxyalkylaminoethane-thiosulfuric acid (water soluble) class of compounds. It was desired to prepare these compounds because they had appeared promising as mercaptans. This problem was resolved, as predicted, and the temporary decrease in the rate of acquisition of compounds was reversed. The Bunte salts corresponding to the promising polyhydroxyalkylamino mercaptans proved disappointing. The synthesis of polyhydroxyalkyl Bunte salts has, therefore, been discontinued and a select number of the corresponding compounds in the mercaptan series have been selected to be synthesized as the phosphorothioates. This is now underway but, as in the case of the shift of emphasis from the mercaptans to the thiosulfates, the shift in emphasis from the thiosulfates to the phosphorothioates has introduced purification problems. These are being worked out but difficulties still exist. This problem is perhaps somewhat more difficult than the Bunte salt purification because of the relative instability of the phosphorothioate group.

A great deal of emphasis has been placed on aziridine chemistry during this last year. The expertise of one of our contractors in the aziridine field has opened up a route to a great many compounds that would be difficult to achieve by other means. The N-aminoalkyl-aziridines will open the way to a whole series of weakly basic nitrogen

functions any given number of carbons away from the nitrogen of the aminoethanethio moiety. These structures have eluded us for some time because of the synthetic difficulties by other routes. In addition, a novel ring opening for aziridines has been discovered which has increased the output of pure compounds, either Bunties or phosphorothioates, tremendously.

In the lipid soluble series the active combination of a ring separated from an aminoethylthio moiety by several methylene groups continues to be investigated in an attempt to find the optimum combination of structural elements. Thus, the size of the ring, the number of methylene groups from the nitrogen atom, the number and type of substituents on the ring, and the effect of sulfur or oxygen interruption of the methylene chain have been looked at. An extremely interesting variation which provided a compound with a very high protective index, WR 3562, indicated that putting the ring on the second carbon of a chain of four may have a salutary effect and this variation is being incorporated in some of the new structures being synthesized.

Because the lipid soluble compounds are poorly absorbed orally, as opposed to the water soluble ones, structures are being designed incorporating features of both. Thus, one or two hydroxyl groups are being incorporated on an alkyl chain so that the ratio of hydroxyls to methylenes is relatively low as opposed to the relatively high ratio that confers water solubility. In addition, it is planned to attempt to block some of the hydroxyls in this type of structure in order to confer more lipid solubility. Approaching from the other side, work is in progress on the synthesis of compounds containing water soluble heterocyclic rings, several methylenes removed from the nitrogen of the aminoethylthio moiety, thus, increasing the water solubility but retaining the same general structure, of the lipid soluble compounds of the type with carbocyclic or aromatic rings down the chain. Hopefully, this will increase absorption on oral administration. This structure variation has, unfortunately, introduced purification problems in the synthesis of this type of compound and at this writing these problems have not been surmounted.

Compounds with the basic nitrogen incorporated in an amidine structure continue to be of interest, but because of the relatively small effort in this area the submission of compounds has been slow. It is planned to continue the synthesis of the amidines, making careful selection of the ones to be synthesized.

Studies on new masking groups for sulfur are continuing with several new types submitted. However, at the present time, these are prototype compounds rather than those that would be expected to be of most interest. The latter should be forthcoming during the coming year. A number of additional analogs related to o-aminoethyl-dithiobenzoic acid have been prepared because of the unexpected activity of this compound. It would appear that the most active compounds in this series are those with an acidic function on the ring ortho to the sulfur atom although others have shown some activity.

The work on chelates and polymers has essentially come to an end. It is unlikely that work in this area will be rejuvenated unless some unexpected lead turns up in the screening program.

The two contract-supported preparations laboratories for the synthesis of larger amounts of promising compounds or important intermediates continue to operate.

The so-called no dollar agreement which facilitates the receipt of unpatented compounds for testing continues to be of value. There are currently twenty-seven companies subscribing to this agreement.

TABLE I

Contract Status - June 1965

| <u>Status</u> | <u>Number of Contracts</u> |
|--|----------------------------|
| Presently active | 13 |
| Discontinued during FY 65 | 3 |
| Scheduled for termination during FY 66 | 5 |
| Pending | 1 |
| Projected active - end of FY 66 | 9 |

TABLE II

Contract Breakdown - June 1965

| <u>Type</u> | <u>Number of Contracts</u> | <u>% of Total</u> | <u>% Total Dollars</u> |
|----------------|----------------------------|-------------------|------------------------|
| Academic | 8 | 62 | 18.6 |
| Industry | 3 | 23 | 59.5 |
| Research House | 2 | 15 | 21.8 |

TABLE III

Approximate Compound Breakdown - FY 65

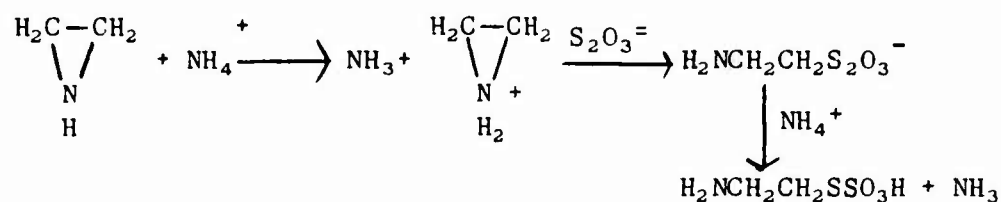
| <u>Type</u> | <u>Number of Compounds</u> | <u>Cost per Compound</u> |
|----------------|----------------------------|--------------------------|
| Academic | 50 | \$ 2,003 |
| Industry | 166 | 2,096 |
| Research House | 88 | 1,449 |

2. WRAIR Medicinal Chemistry Laboratory

Twenty-one compounds were submitted for testing as potential anti-radiation drugs, 18 of which are not described in the chemical literature.

Several techniques were developed or improved for the synthesis of aminoalkylthiosulfuric acids (so called amino Bunte salts). A brief description of these methods follows.

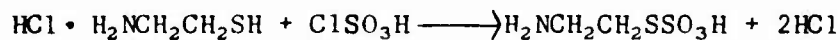
The ring-opening of protonated aziridines with sodium thiosulfate sometimes results in isolation difficulties due to the similar solubilities of the aminoalkylthiosulfuric acids and the salts formed as by-products. Ammonium thiosulfate was found to be more desirable in some ways as a ring-opening reagent than sodium thiosulfate. Use of the ammonium salt made the usual acidification step unnecessary and resulted in the formation of ammonia as an easily removable by-product. By performing the reaction in methanol, the unreacted ammonium thiosulfate could be separated by filtration at the end of the reaction period.



The conversion of aminoalkylthiols to Bunte salts involves the oxidation of the thiol to the disulfide followed by cleavage of the disulfide with sulfite to form 1 equiv. of thiol and 1 equiv. of thiosulfate.

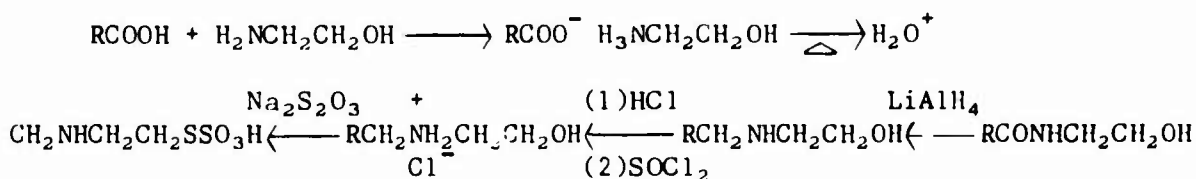


A one-step conversion was found in which the aminoalkylthiol hydrochloride, suspended in cold ether, is treated with chlorosulfonic acid.



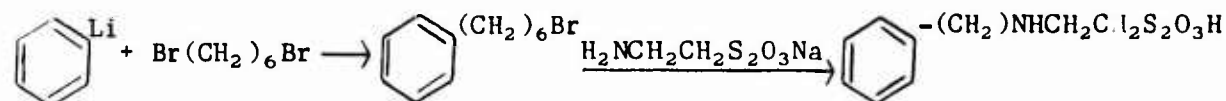
Additional work is in progress to improve the yields of the Bunte salts obtained.

A method was developed utilizing aliphatic and aromatic carboxylic acids to provide potential alkyl and aralkyl groups for N-substituted 2-aminoethanethiosulfuric acids. The carboxylic acid was heated with 1 equiv. of 2-aminoethanol at 160-200°. After the calculated quantity of water formed was collected in a Dean-Stark tube, the crude 2-hydroxyethylamide (which frequently crystallized on cooling) was reduced in tetrahydrofuran solution with lithium aluminum hydride.



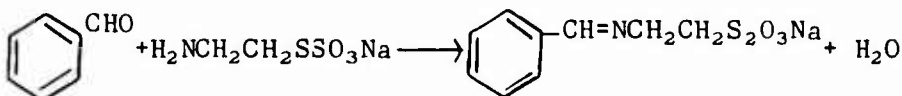
The aminoalcohol, thus formed, was treated first with anhydrous hydrogen chloride and then, thionyl chloride to give the aminoethylchloride hydrochloride. The reaction of this product with 1 equiv. of sodium thiosulfate in aqueous or aqueous ethanolic solution gave the desired amino Bunte salt.

Phenylalkylaminoethanethiosulfuric acids have shown interesting anti-radiation properties. While many of the phenylalkyl halides are commercially available, phenylhexyl bromide is not. A coupling reaction between 1,6-dibromohexane and phenyllithium was developed which provided the desired product. This technique probably



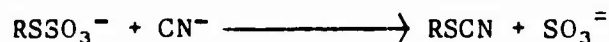
has general applicability to the synthesis of other aralkyl halides.

It was found that Schiff bases can be made from sodium aminoethylthiosulfate and several aromatic, but not aliphatic, aldehydes. These water-soluble compounds have the property of reverting

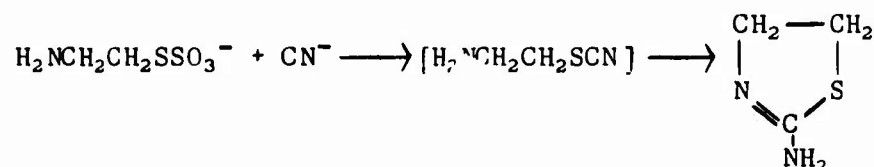


immediately in acid solution to the starting aminoethylthiosulfuric acid and aldehyde. These Schiff bases, therefore, may prove to be useful for oral administration.

The action of cyanides on alkylthiosulfates has been known to give alkylthiocyanates and sulfite ion. In applying this

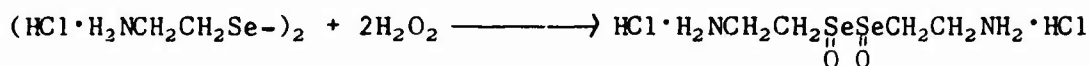


reaction to aminoethylthiosulfates, the aminoethylthiocyanate intermediate cyclized, as anticipated, to give 2-aminothiazoline (90% yield.) This reaction has been tried with a number of amino Bunte salts to give



the corresponding aminothiazolines (or 2-aminothiazolidinines) where the amino group of the starting Bunte salt is 1° or 2° and the corresponding aminoalkylthiocyanate where the original amino group is 3°. A few of the aminothiazolines show encouraging anti-radiation properties and may account for Russian interest in this area.

The product obtained from the oxidation of selenocystamine dihydrochloride with 2.2 equiv. of hydrogen peroxide has been studied by n.m.r. Spectra of the dioxide and its free base indicate molecular symmetry suggesting a diselenoxide structure. While investigators in Se chemistry have tried to isolate intermediates in the oxidation of diselenides to seleninic acids, none, to our knowledge, have been successful. Bis-(2-aminoethyl) diselenoxide dihydrochloride is believed, there, to be the first compound of its type to be



synthesized and isolated. X-ray diffraction studies of the material are now under way.

The oxidation of cystamine dihydrochloride to the monoxide, 2-aminoethyl 2-aminoethanethiolsulfinate dihydrochloride, has been accomplished through the use of m-chloroperbenzoic acid. Unlike cystamine or the corresponding thiolsulfonate, it does not

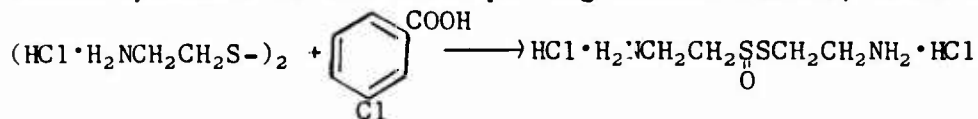
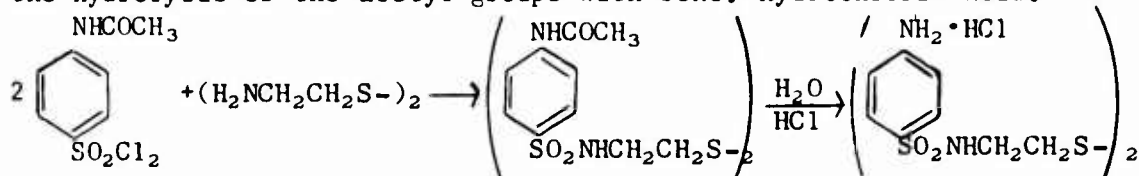


exhibit good anti-radiation properties.

A sulfonamide derivative of cystamine was made by the reaction of cystamine and N-acetylsulfanilyl chloride, followed by the hydrolysis of the acetyl groups with conc. hydrochloric acid.



A fluidextract of the Manchurian plant, Eleutherococcus senticosus has been reported by I.I. Brekhman (Izv. Sibir. Otdel. Akad. Nauk. SSSR, 9, 113 (1960)) to possess anti-radiation activity. Some of the crude plant material was acquired from Dr. Bruce W. Halstead, Director, World Life Research Institute, Colton, California and extracted. Testing of the fluid extract of the Eleutherococcus is now under way.

Papers published:

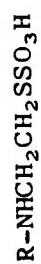
- (1) D. L. Klayman and W. F. Gilmore; The Synthesis of N-Substituted 2-Aminoethanethiosulfuric Acids, J. Med. Chem., 7, 823 (1964).
- (2) D. L. Klayman, J. D. White and T. R. Sweeney; Unsymmetrical Disulfides from an Amino Bunte Salt, J. Org. Chem., 29, 3737 (1964).

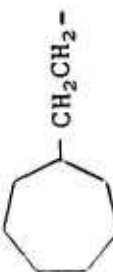
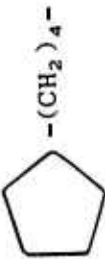
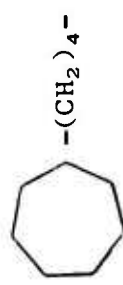

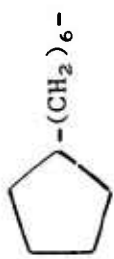
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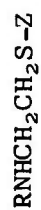
- (3) D. L. Klayman; Synthesis of Aminoethyl Selenium Compounds, J. Org. Chem. (July, 1965).
- (4) D. L. Klayman, J. W. Lown and T. R. Sweeney; Nucleophilic Displacement of Bromide by Thiosulfate from 1,2-Aminobromopropanes, J. Org. Chem. (July, 1965).

Papers in preparation:

- (5) D. L. Klayman, W. F. Gilmore and T. R. Sweeney; The Opening of Aziridines with Ammonium Thiosulfate.
- (6) D. L. Klayman, J. W. Lown and P. K. Iber; The Oxidation of Selenocystamine to the Diselenoxide.
- (7) D. L. Klayman; The Action of Cyanide on Aminoalkylthiosulfates.



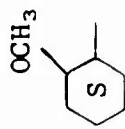
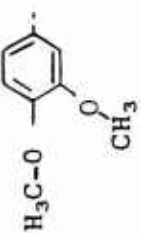


| WR | R | LD ₅₀ | Test Dose | % Survival | Index |
|------|---|------------------|---------------------------|----------------|----------------------|
| 3341 |  | 80 | 50 25 | 40 0 | 2.2 0 |
| 3298 |  | 175 | 75 37.5 18 | 93 100 0 | 4.5 5.00 0 |
| | | 938(po) | 500(60 pre) " (30 pre) | 33 20 | 2.5 2.2 |
| 3561 |  | 15 | 5 2.5 | 87 13 | 5.6 6.7 |
| 3613 |  | 150 | 50 25 12.5 | 80 87 53 | 5.4 11.2 18.36 |
| 3302 |  | 10 | 5 2.5 | 80 20 | 3.6 3.0 |
| | | >1000(po) | 800(60 pre) " (30 pre) | 47 47 | 5.9 5.9 |

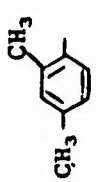
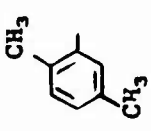

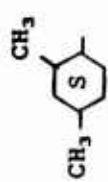
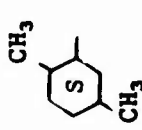



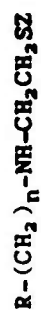
| WR | R | Z | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|-------------------------|------------------|----------------------|------------|-------|
| 3823 | $\text{CH}_2=\text{CH}-(\text{CH}_2)_3-$ | SO_3H | 225 | 100 | 0 | 0 |
| 3358 | $(\text{CH}_3)_2\text{CH}(\text{CH}_2)_3\underset{\text{CH}_3}{\text{CH}}\text{CH}_2\text{CH}_2-$ | SO_3H | 125 | 30 | 87 | 7.7 |
| | | | | 15 | 80 | 14.9 |
| | | | | 8 | 33 | 20.8 |
| | | | | 4 | 0 | 0 |
| 3679 | | PO_3H_2 | >1000(po) | 1000(60 pre) | 0 | 0 |
| | | | | 1000(30 pre) | 7 | 1.1 |
| | | | | 25 (14 day) | 7 | 2.7 |
| | | | | 12.5 | 0 | 0 |


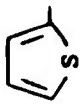



| WR | R | LD ₅₀ | TEST DOSE | % SURVIVAL | INDEX |
|------|---|------------------|--|--------------------------------|--------------------------------------|
| 3299 |  | 250 | 150 75 | 27 0 | 2.1 0 |
| 3050 |  | 100 | 30 15 8 4 | 87 80 7 0 | 6.2 12.0 21.3 0 |
| | Depressant | >1000(po) | 1000 (15 pre) (30 pre) (60 pre) (120 pre) 500 (30 pre) 250 (30 pre) | 7 87 67 13 20 0 | 1.7 1.9 1.7 1.1 2.4 0 |
| 3614 |  | 175 | 50 25 12.5 6 | 93 93 7 7 | 6.8 13.5 14.9 30 |
| 3338 |  | 350 | 150 75 | 20 13 | 2.8 5.3 |







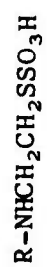
| MR | R | LD ₅₀ | Test Dose | % Survival | INDEX |
|------|---|------------------|--|----------------------|--------------------------|
| 3340 |  | 38 | 15 7.5 1000 (30 pre) " (60 pre) | 73 7 0 0 | 4.4 8.6 0 0 |
| 3337 |  | 20 >1200 (po) | 10 5 1000 (60 pre) " (30 pre) | 73 7 0 0 | 3.5 6.8 0 0 |
| 3342 |  | 15 >1000 (po) | 5 2.5 500 (60 pre) " (30 pre) | 60 13 57 53 | 4.8 6.8 3.1 3.0 |
| 3564 |  | 30 | 20 10 | 73 47 | 2.6 4.4 |
| 3339 |  | 25 | 15 7.5 | 83 33 | 3.0 4.4 |
| 3558 |  | 6 | 3 1.5 | 13 0 | 2.3 0 |

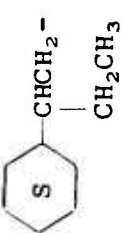
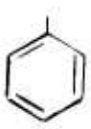
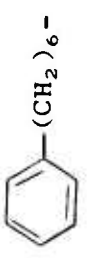
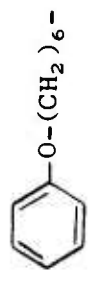
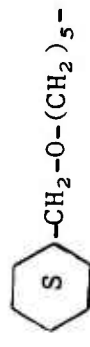


| WR | R | n | Z | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|---|-------------------|------------------|----------------------|------------|-------|
| 3818 |  | 1 | H | > 200 | 150 | 0 | 0 |
| 3085 |  | 4 | SO ₃ H | 350 | 200 | 67 | 2.9 |
| | | | | | 100 | 60 | 5.6 |
| | | | | | 50 | 7 | 7.5 |
| | | | | | 25 | 7 | 15.0 |
| 3609 |  | 4 | SO ₃ H | 300 | > 1600 (po) | 0 | 0 |
| | | | | | 1600 (60 pre) | 0 | 0 |
| | | | | | 1600 (30 pre) | 0 | 0 |
| | | | | | 150 | 7 | 2.1 |
| | | | | | 75 | 7 | 4.2 |



| VR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|--|------------------|----------------------|------------|------------|
| 3612 |  | 20 | 10 5 | 27 20 | 2.5 4.8 |
| 3611 |  | 25 | 15 (tox) 7.5 | 0 27 | 0 4.2 |
| 3566 |  | 15 | 8 4 | 53 20 | 2.9 4.5 |
| 3560 |  | 18 | 10 | 0 | 0 |



| WR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3562 |  | 300 | 100 | 80 | 5.4 |
| | | | 50 | 80 | 10.8 |
| | | | 25 | 53 | 18.4 |
| | | | 12.5 | 20 | 28.8 |
| 3552 |  | >800 | 400 | 0 | 0 |
| 3313 |  | 37.5 | 25 | 27 | 1.9 |
| | | | 12.5 | 20 | 3.6 |
| 3821 |  | 500 | 50 | 0 | 0 |
| | | | 25 | 0 | 0 |
| 3567 |  | 18 | 12.5 | 47 | 2.1 |
| | | | 6.25 | 27 | 3.6 |

Depressant



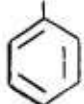
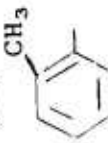
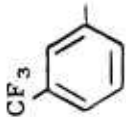
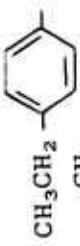
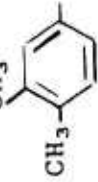
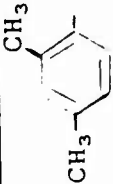
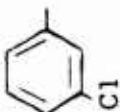
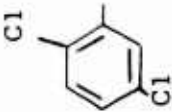

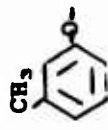


| WR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3305 |  | 275 | 200 | 0 | 0 |
| 3087 |  | 160 | 50 | 69 | 5.4 |
| | | | 25 | 78 | 11.4 |
| | | | 12 | 20 | 15.9 |
| | | | 6 | 0 | 0 |
| | | > 1200 (po) | 1000 (30) | 0 | |
| | | | (60) | 0 | |
| 3335 |  | 45 | 30 | 80 | 2.7 |
| | | | 15 | 0 | 0 |
| | | > 1200 (po) | 1000 | 0 | |
| 3565 |  | 30 | 5 | 0 | 0 |
| 3343 |  | 45 | 30 | 87 | 2.8 |
| | | | 15 | 47 | 4.4 |
| | | > 1200 (po) | 1000 | 0 | |

Figure 8





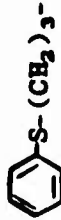
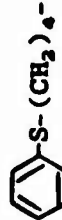


| WR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3336 |  | 20 | 10 | 87 | 3.7 |
| | | | 5 | 40 | 5.6 |
| | | > 1000 (po) | | | |
| 3819 |  | 150 | 100 | 73 | 2.6 |
| | | | 50 | 73 | 5.2 |
| | | | 25 | 53 | 9.2 |
| | | | 12.5 | 7 | 12.98 |
| 3122 |  | 150 | 25 | 93 | 11.6 |
| | | | 12.5 | 33 | 16.0 |
| | | > 2000 (po) | 1000 | 0 | |
| 3610 |  | 38 | 15 | 53 | 3.9 |
| | | | 7.5 | 0 | 0 |



| WR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|--|------------------|----------------------|------------|------------|
| 3301 |  | 125 | 75 37.5 | 40 27 | 2.3 4.2 |
| 3304 |  | 115 | 75 37.5 | 13 0 | 1.7 0 |
| 3300 |  | 175 | 75 37.5 | 20 0 | 2.8 0 |

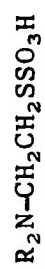
R-NHCH₂CH₂SH


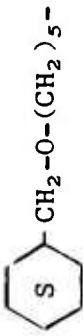
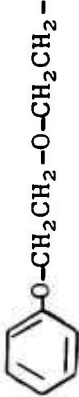
| NR | R | LD ₅₀ | Test Dose | % Survival | Index |
|------|---|------------------|--------------------------------------|-------------------|------------------|
| 3233 |  | | 40 20 150(15pre) 150(30pre) | 53 0 0 0 | 0 0 0 0 |
| 3307 |  | 125 | 75 37.5 | 13 0 | 1.9 0 |
| 3354 |  | 125 | 75 | 0 | 0 |
| 3583 |  | 88 | 50 25 | 20 0 | 2.0 0 |
| 3697 |  | 150 | 50 | 0 | 0 |
| 3431 |  | 75 | 50 | 0 | 0 |

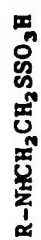
$\text{CH}_3(\text{CH}_2)_8\text{R}$
 SO_3H

| NR | R | R ¹ | LD ₅₀ | Test Dose | % Survival | Index |
|------|--|-----------------------|------------------|---------------------------------------|-------------|-------------|
| 3423 | $\text{CH}_3(\text{CH}_2)_7-$ | SO_3H | 350 >3000 | 200 1500 (60 pre) 1500 (30 pre) | 0 0 0 | 0 0 0 |
| 3605 | $\text{CH}_3(\text{CH}_2)_7-$ (formyl) | SO_3H | 300 | 200 100 | 7 0 | 1.6 |
| 3539 | $\text{CH}_3(\text{CH}_2)_6-$ | H | 375 | 100 | 0 | 0 |
| 3432 | $\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_2-$ | H | 350 | 250 | 0 | 0 |
| 3698 | $\text{CH}_3(\text{CH}_2)_3\text{OCH}_2\text{CH}_2-$ | H | 350 | 100 | 0 | 0 |
| 3540 | $\text{CH}_3(\text{CH}_2)_8-$ | H | 250 | 100 | 0 | 0 |

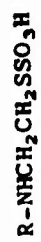
Figure 12

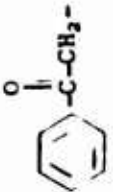



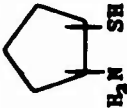



| WR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3820 |  | > 2000 | 100 (15 day) | 0 | 0 |
| | | | 50 | 0 | 0 |
| 3563 |  | 125 | 25 | 0 | 0 |
| 3303 |  | 450 | 400 | 0 | 0 |

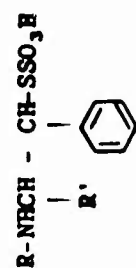


| WR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|--|------------------|----------------------|------------|-------|
| 3553 | $\begin{array}{c} O \\ \\ CH_3-O-C-CH_2- \end{array}$ | > 800 | 400 | 0 | 0 |
| 3554 | $\begin{array}{c} O \\ \\ CH_3CH_2-O-C-CH_2CH- \\ \\ CH_3 \end{array}$ | > 800 | 800 | 0 | 0 |
| 3680 | $\begin{array}{c} O \\ \\ CH_3-O-C-CH-CH_2- \\ \\ CH_3-O-C-CH_3 \\ \\ O \end{array}$ | > 2000 | 1500 | 0 | 0 |
| 3597 | HOOC(CH ₂) ₄ - | > 800 | 800 | 0 | 0 |
| 3133 | (HOOCCH ₂) ₂ (Mercaptan) | > 1000 | 400 | 0 | 0 |



| LR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|----------|
| 360f | HO ₃ S(CH ₂) ₃ NHCH ₂ CHSH CH ₃ | >2000 | 2000 1000 | 20 0 | 1.2 0 |
| 3683 |  | 300 | 75 | 0 | 0 |
| 3682 |  | 375 | 200 | 0 | 0 |

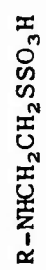
| NR | COMPOUND | LD ₅₀ | Test Dose | % Survival | Index |
|------|---|------------------|------------|------------|------------|
| 3542 |  | 325 | 250 125 | 73 27 | 2.3 3.3 |
| 3349 |  | 250 | 75 | 0 | 0 |
| 3311 |  | 400 | 200 100 | 43 0 | 2.9 0 |
| 3312 |  | >500 | 75 | 0 | 0 |



| MR | R | R' | LD ₅₀ | Test Dose | % Survival | Index |
|------|---|-----------------|------------------|-----------|------------|-------|
| 3575 | H-dl-erythro | CH ₃ | 200 | 100 | 0 | 0 |
| 3574 | CH ₃ -d(-)erythro | CH ₃ | 500 | 300 | 0 | 0 |
| 3576 | CH ₃ -d(+)threo | CH ₃ | 125 | 50 | 0 | 0 |
| 3551 | (CH ₃) ₂ CHCH ₃ - | H | 600 | 400 | 0 | 0 |
| 3550 | CH ₃ (CH ₂) ₃ - | H | 300 | 100 | 0 | 0 |
| 3549 | (CH ₃) ₂ CH- | H | 200 | 100 | 0 | 0 |
| 3548 | CH ₃ CH ₂ CH ₃ - | H | 300 | 100 | 0 | 0 |

R-NHCH₂CH₂SH

| WR | R | LD ₅₀ | TESTED DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|--|------------------|--|--------------------|-----------------------------|
| 3100 | HO(CH ₂) ₃ NHCH ₂ CH ₂ - | 350 | 400 (tox) 300 200 | 27 20 20 | 1.1 1.3 2.1 |
| 3215 | HOCH ₂ CHCH ₂ OCH ₂ CH ₂ - OH | 1625 | 1000 500 250 1000 (15 pre) 1000 (30 pre) | 93 40 0 7 | 3.1 4.5 0 3.2 0 |
| 3094 | HOCH ₂ CHCH ₂ CH ₂ NHCH ₂ CH ₂ - OH | | 800 (tox) 700 400 | 13 13 33 | - - - |
| 3297 | (CH ₃) ₂ C-CHCH ₂ - OH OH | 650 | 200 100 | 7 0 | 3.5 0 |
| 3120 | HOCH ₂ CH ₂ C-CH ₂ - OH CF ₂ CF ₃ | >400 | 200 100 | 13 0 | 2.3 0 |
| 3356 | CH ₃ CH ₂ OCH ₂ CH ₂ - | 175 | 100 | 0 | 0 |



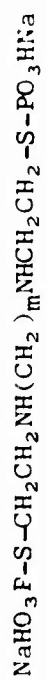
| WR | R | LD ₅₀ | TEST DOSE | % SURVIVAL | INDEX |
|------|--|------------------|-------------|------------|------------|
| 3124 | HOCH ₂ CH ₂ - | 1200 | 800 400 | 27 27 | 1.9 3.8 |
| 3559 | (HOCH ₂) ₂ CH- | 1750 | 1250 625 | 73 40 | 2.4 3.9 |
| 3435 | (HOCH ₂) ₂ C(CH ₂ SH)- | 1000 | 1000 | 0 | 0 |
| 3559 | HOCH ₂ CH ₂ NHCH ₂ CH ₂ - | 2000 | 1000 | 0 | 0 |
| 3604 | HOCH ₂ CH ₂ OCH ₂ CH ₂ - | 900 | 800 400 | 93 20 | 2.2 2.7 |
| 3607 | HOCH ₂ CH(OH)CH ₂ OCH ₂ CH ₂ - | 2000 | 1500 750 | 87 13 | 2.5 2.2 |

R-NHCH₂CH₂SSCH₂CH₂NHR

| WR | R | LD ₅₀ | TEST DOSE | % SURVIVAL | INDEX |
|------|---|------------------|--|--------------------|-------------------------|
| 3601 | HOOC-CH- CH ₃ | 150 | 100 | 0 | 0 |
| 3123 | HOCH ₂ CH ₂ - | 800 | 400 200 1600 (po) 1600 (15 pre) | 73 7 0 0 | 3.5 4.3 0 0 |
| 3348 | HOCH ₂ CH ₂ OCH ₂ CH ₂ - | 750 | 750 | 0 | 0 |
| 3347 | HOCH ₂ CHCH ₂ NHCH ₂ CH ₂ - OH | 700 | 600 300 | 7 0 | 1.2 |
| 3568 | (HOCH ₂) ₂ CH- | 1250 | 800 400 160 80 | 93 63 7 0 | 3.0 5.1 8.3 |
| 3346 | HO(CH ₂) ₆ NHCH ₂ CH ₂ - | 175 | 100 | 0 | 0 |
| 3326 | HOCH ₂ CH ₂ NHCH ₂ CH ₂ - | 450 | 400 (toxic) 200 | 0 13 | 0 2.5 |
| 3606 | HOCH ₂ CHCH ₂ -O-CH ₂ CH ₂ - OH | 1250 | 1000 500 250 125 | 93 53 7 0 | 2.4 3.8 5.35 0 |
| 3344 | CH ₃ (CH ₂) ₉ NHCH ₂ CH ₂ - | 25 | 15 | 0 | 0 |

| WR | COMPOUND | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|--|----------------------|--------------------------|
| 3599 | $\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{H}_2\text{N}-\text{C}-\text{C}-\text{NH}(\text{CH}_2)_3\text{NHCH}_2\text{CH}_2\text{SSO}_3\text{H} \end{array}$ | 700 | 600 300 | 7 0 | 1.2 |
| 3350 | $\begin{array}{c} \text{O} \\ \\ \text{[C-NH(CH}_2)_3\text{NHCH}_2\text{CH}_2\text{SSO}_3\text{H}]_2 \end{array}$ | 300 | 200 | 0 | 0 |
| 3600 | $\begin{array}{c} \text{O} \\ \\ \text{[C-NH(CH}_2)_3\text{NHCH}_2\text{CH}_2\text{SH}]_2 \end{array}$ | 38 | 25 12.5 | 33 0 | 2.0 0 |
| 3693 | $\begin{array}{c} \text{O} \\ \\ \text{C}_6\text{H}_4-\text{NHC-NH(CH}_2)_3\text{NHCH}_2\text{CH}_2\text{SH} \end{array}$ | 150 | 50 | 0 | 0 |
| 3598 | $\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{H}_2\text{NC-C-NH(CH}_2)_3\text{NHCH}_2\text{CH}_2\text{SH} \end{array}$ | 900 PO > 1000 | 400 200 850 (30 pre) 850 (15 pre) | 53 40 47 30 | 2.7 4.9 1.7 1.6 |

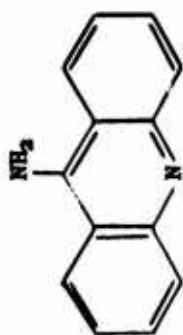
Figure 21



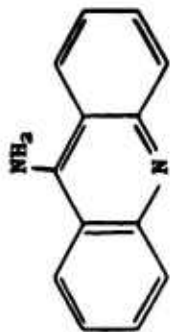
| WR | m | LD ₅₀ | Test Dose | % Survival | Index |
|------|---|------------------|-----------|---------------|-------|
| 3694 | 3 | 400 | 250 | 90 | 3.1 |
| | | | 125 | 73 | 5.5 |
| | | | 63 | 33 (15th day) | 5.5 |
| | | | 31.5 | 0 | 0 |
| 3695 | 5 | 125 | 50 | 7 | 2.7 |
| | | | 25 | 7 | 5.4 |
| 3696 | 6 | 38 | 25 | 7 | 1.6 |
| | | | 12.5 | 0 | 0 |

940 Figure 22

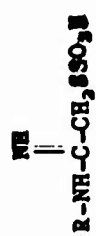
| NR | LD ₅₀ | DRUG DOSE | TIME PRE RAD (min) | % SURVIVAL |
|--------------|------------------|---------------|--------------------------|-----------------|
| 2721 | 1000 | 600 | 60 180 300 | 100 90 10 |
| | | | | |
| 2921 | | 25 | 60 180 300 | 40 0 10 |
| | | | | |
| 2721 2921 | | 600 } 25 } | 60 180 300 | 100 90 60 |


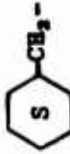


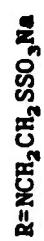
WR 2921






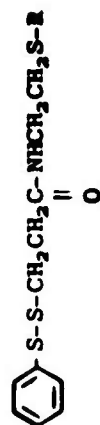
| No. of Animals | Injected Dose mg/kg | No. of Injections | Time Pre-Radiation (hours) | % 5-day Deaths | % Survival |
|----------------|---------------------|-------------------|----------------------------|----------------|------------|
| 12 | 25 | 4 | 72, 48, 24, 1 | 83 | 0 |
| | " | " | " | 18 | 0 |
| 9 | 25 | 3 | 48, 24, 1 | 33 | 0 |
| | " | " | " | 27 | 0 |
| 10 | 25 | 2 | 24, 1 | 30 | 0 |
| | " | " | " | 25 | 30 |
| 15 | 15 | 1 | 1 | 0 | 0 |
| | " | " | 1/2 | 0 | 0 |
| | 30 | " | 1/4 | 0 | 0 |
| 12 | 25 | 6 | 60, 48, 36, 24, 12, 1 | 83 | 0 |
| 10 | 25 | 5 | 48, 36, 24, 12, 1 | 20 | 0 |
| 10 | 25 | 4 | 36, 24, 12, 1 | 0 | 20 |


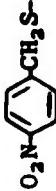





| NR | R | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3319 |  | 75 | 50 | 67 | 2.5 |
| | | | 25 | 13 | 3.4 |
| 3537 |  | 60 | 15 | 47 | 5.9 |
| | | | 7.5 | 7 | 8.6 |
| 3308 | $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2-$ | 70 | 40 | 53 | 2.7 |
| | | | 20 | 13 | 4.0 |
| | | 375 (po) | 125 (60 pre) | 40 | 4.2 |
| | | | 125 (30 pre) | 27 | 3.8 |





| WR | R | LD50 | Test Dose | % Survival | Index |
|------|---|------|------------|------------|------------|
| 3359 |  | >800 | 600 300 | 93 0 | 2.6 0 |
| 3361 |  | 800 | 600 300 | 40 7 | 1.9 2.8 |
| 3360 |  | 800 | 600 300 | 73 0 | 2.3 0 |



| WR | R | LD ₅₀ | Test Dose | % Survival | Index |
|------|--|------------------|-----------|------------|-------|
| 3324 | HOOCCH ₂ CH ₂ S- | 150 | 50 | 0 | 0 |
| 3322 | CH ₃ -  S- | 125 | 25 | 0 | 0 |
| 3320 | O ₂ N-  CH ₂ S- | >400 | 400 | 0 | 0 |
| 3321 |  S-SCH ₂ CH ₂ C(=O)NHCH ₂ CH ₂ S- | >200 | 200 | 0 | 0 |
| 3323 |  CH ₂ SSCH ₂ CH ₂ C(=O)NHCH ₂ CH ₂ SSCH ₂ -  | 150 | 25 | 0 | 0 |

| Index | Compound | LD ₅₀ | Test Dose | % Survival | Index |
|-------|---|------------------|-----------|------------|-------|
| 3556 | $ \begin{array}{c} \text{O} \quad \text{NH} \\ \quad \\ \text{CH}_3\text{CH}_2\text{O}-\text{C}-\text{N}-\text{O}-(\text{CH}_2)_3\text{S}-\text{C}-\text{NH}_2 \\ \\ \text{CH}_3 \end{array} $ | 250 | 200 | 0 | 0 |
| 3318 | $ \begin{array}{c} \text{O} \quad \text{NH} \\ \quad \\ \text{CH}_3\text{CH}_2\text{O}-\text{C}-\text{N}-\text{CH}_2-(\text{CH}_2)_3\text{S}-\text{C}-\text{NH}_2 \\ \\ \text{OCH}_2\text{CH}_3 \end{array} $ | 125 | 75 | 0 | 0 |
| 3686 | $ \begin{array}{c} \text{O} \quad \text{NH} \\ \quad \\ \text{CH}_3\text{CH}_2\text{O}-\text{C}-\text{N}-(\text{CH}_2)_3\text{S}-\text{C}-\text{NH}_2 \\ \\ \text{OCH}_2\text{CH}_3 \end{array} $ | 175 | 100 | 0 | 0 |
| 3557 | $ \begin{array}{c} \text{O} \\ \\ \text{CH}_3\text{CH}_2\text{O}-\text{C}-\text{N}-(\text{CH}_2)_3\text{SH} \\ \\ \text{OCH}_2\text{CH}_3 \end{array} $ | 800 | 500 | 0 | 0 |

| WR | COMPOUND | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|--|------------------|----------------------|-----------------|--------|
| 3538 | $\text{CH}_3\text{-}$  -S-S-C(=S)-NHCH_3 | 300 | 100 | 0 | 0 |
| 3596 | $\text{CH}_3\text{-C(=O)-NHCH}_2\text{CH}_2\text{-S-S-C(=S)-NHCH}_3$ | 60 | 25 | 0 | 0 |
| 3699 | $\left[\text{CH}_3\text{-C(=O)-NHCH}_2\text{CH}_2\text{S} \right]_2\text{S}$ | 300 | 50 25 | 0 (14 day) 0 | 0 0 |
| 3554 | $\text{CH}_3\text{-C(=O)-NHCH}_2\text{CH}_2\text{-S-S-C(=O)-}$  | 75 | 25 | 0 | 0 |



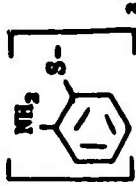
| NR | COMPOUND | LD ₅₀ | TESTED DOSE | % SURVIVAL | INDEX |
|------|---|------------------|-------------|-----------------|----------|
| 3629 | $\begin{array}{c} \text{H}_2\text{NCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{NH}_2 \\ \downarrow \\ \text{O} \end{array}$ | 250 | 100 50 | 7 (23 day) 0 | 2.7 0 |
| 3353 | $(\text{HO}_2\text{SSCH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2)_2\text{SO}_2$ | 550 | 400 | 0 | 0 |
| 3351 | $(\text{HSCCH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2)_2\text{SO}_2$ | 300 | 150 | 0 | 0 |

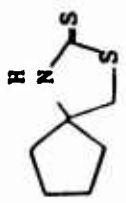
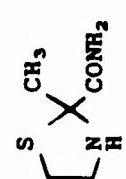
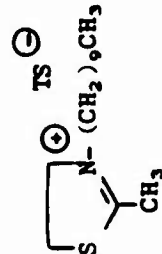
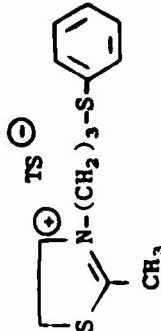
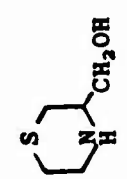
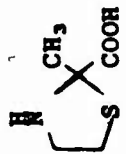
957

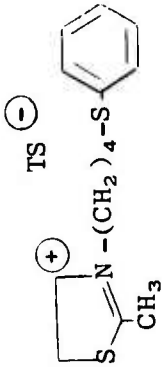
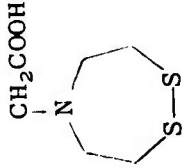
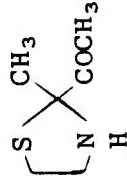
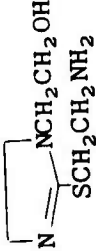
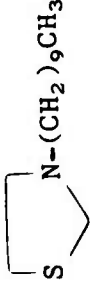
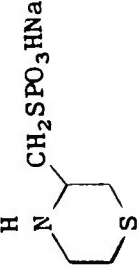
Figure 30

| WR | CHELATE | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|--|------------------|----------------------|------------|-------|
| 3294 | $[\text{SCH}_2\text{CH}_2\text{NHCH}_2\text{COO}]_2\text{Zn}_2$ | 37 | 25 | 0 | 0 |
| 3295 | $[\text{SCH}_2\text{CH}_2\text{NHCH}_2\text{COO}]_2\text{Cd}$ | 60 | 15 | 0 | 0 |
| 3296 | $[\text{HOOCCH}_2\text{NHCH}_2\text{CH}_2\text{S}] [\text{Pd O Cl}]$ | 125 | 5 | 0 | 0 |

MISCELLANEOUS

| WR | COMPOUND | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3547 | $(\text{CH}_3\text{CH}_2\text{O})_2\text{P}(\text{S})\text{CH}_2\text{CH}_2\text{NH}_2$ | 3 | 1 | 0 | 0 |
| 3136 | $[(\text{HOOCCH}_2)_2\text{NCH}_2\text{CH}_2\text{SCH}_2-]_2$ | > 800 | 800 | 0 | 0 |
| 3687 |  | 150 | 75 | 0 | 0 |
| 3310 |  | 38 | 25 | 0 | 0 |
| 3543 | $\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{SCN}$ | 30 | 10 | 0 | 0 |
| 462 |  | 125 | 25 | 0 | 0 |

| WR | COMPOUND | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|----------------------|------------|-------|
| 3691 |  | 5 | 3 | 0 | 0 |
| 3692 |  | > 1200 | 1200 | 0 | 0 |
| 3541 |  | 80 | 25 | 0 | 0 |
| 3357 |  | 90 | 60 | 0 | 0 |
| 3355 |  | > 800 | 800 | 0 | 0 |
| 3352 |  | > 800 | 800 | 0 | 0 |

| WR | COMPOUND | LD ₅₀ | TEST DOSE (mg/kg) | % SURVIVAL | INDEX |
|------|---|------------------|---|---------------------|------------------------|
| 3325 |  | 125 | 75 | 0 | 0 |
| 3317 |  | 187 | 100 50 | 40 0 | 2.6 0 |
| 3309 |  | > 1000 | 800 400 | 43 7 | 1.8 2.7 |
| 3306 |  | 125 | 100 | 0 | 0 |
| 2950 |  | 75 | 25 12.5 | 60 0 | 4.8 0 |
| 3690 |  | 225 (po) 375 | 100 (15 pre) 100 (30 pre) 100 (60 pre) 125 | 10 80 60 0 | 3.7 4.1 3.6 0 |

C. Evaluation of the Antiradiation Action of Chemicals

1. The Mouse Program

The mouse test system continues to be the basic program by which chemicals are examined for radioprotective effectiveness. (See Figure 1) A total of 1099 new compounds have been tested during the past year of which 744 were examined on contract at Woodard Research Corporation and 355 were examined at Walter Reed. Figure 2 summarizes the categories of activity. There has been an increase in the absolute as well as relative number of compounds showing good (i.e. over 45% survival) protection against lethal radiation. The percentage of compounds tested having good protective activity is more than double that reported one year ago.

Additional evaluatory tests were performed for compounds exhibiting activity in the initial testing. These include testing at 1/4 and 1/8 of the maximum tolerated dose, evaluation of the duration of effect, and oral administration. The dose reduction factor is determined on compounds that continue to show promise through the secondary tests. The greatest dose reduction factors have been achieved with combinations of drugs. These tests were discussed in greater detail in another portion of this report.

The output of the mouse screening program at Woodard Research Corporation has increased over the last year. The pseudomonas problem which was prevalent a year ago is now under control and testing is currently moving ahead at the rate of 2,000 compounds per year.

2. Bacterial Testing Program

The current rate of testing in the bacterial anti-radiation system will approach a rate of 3,000 compounds per year within the next 6 month period. 600 compounds have been tested in the past year.

Figure 1

SUMMARY OF MOUSE TESTING

WRAIR

| | |
|---|------------|
| Total number of new agents tested | 355 |
| Special retests | 20 |
| Agents tested at 1/4 and 1/8 doses | 31 |
| Agents tested by oral administration | 46 |
| Agents tested for duration of effect | 9 |
| Agents tested by subcutaneous administration | 3 |
| Agents tested against sublethal radiation | 3 |
| Agents tested for dose reduction | 21 |
| Agents tested in combination (primarily for dose reduction) | 65 |
| Long term administration in diet | 12 |
| Agents tested against nitrogen mustard | 16 |
| Combination tested against nitrogen mustard | 1 |
| Agents tested against Neutron/gamma exposure | 20 |
| Combinations tested against Neutron/gamma exposure | 10 |
| Agents run on special tests | 29 |
| Total | <u>641</u> |

Woodard Research Corporation

| | |
|---------------------|-----|
| New agents tested | 744 |
| Known agents tested | 65 |

Figure 2

RESULTS ON NEW COMPOUNDS TESTED

| <u>WRAIR</u> | <u># of Compounds</u> | <u>%</u> |
|--|-----------------------|----------|
| Good protection vs Lethal Radiation (over 45% survival) | 76 | 21.4 |
| Fair protection vs LR (26-44% survival) | 20 | 5.6 |
| Some protection vs LR (less than 26%) | 59 | 16.6 |
| No protection vs LR | 200 | 56.4 |
| <u>Woodard Research Corporation</u> | | |
| Greater than 33% survival vs LR | 110 | 14.8 |
| 17% survival or no protection | 644 | 85.2 |

3. Large Animal Testing

The toxicity and radioprotective capability of selected chemicals has been studied in the dog and in the rhesus monkey. All compounds included in this report were effective in protecting against radiation in the rodent test system at 1/4 or less of the maximum tolerated dose. The dog and monkey, however, tolerated only 1/4 to 1/2 the mouse dose, with the exception of compound WR 1607 in which the dog and monkey tolerance exceeded the mouse tolerance and WR 2529 in which the dog was particularly sensitive compared to the mouse and monkey. The details of all experimental procedures utilized in the studies reported here may be found in the Annual Report dated July 1963 - June 1964.

The results of antiradiation drug tolerance studies in the dog are included in Tables 1 and 2. Compound WR 1607, an agent previously demonstrated to be effective in protecting dogs against radiation at 10 mg/kg when given intravenously was administered orally as a stable water in oil emulsion in dimethylsulfoxide, octanoic acid and cottonseed oil without evidence of absorption based on observation of clinical signs. Experiments with simple aqueous suspensions also failed to yield positive evidence of gastrointestinal absorption as previously reported.

Compound WR 2691 in the dog produced prompt emesis, prolonged moderate hypertension, severe dyspnea, convulsions, salivation and diarrhea as dominant features of the syndrome following intravenous administration. Violent convulsant activity was observed at doses in excess of 20 mg/kg.

Compounds 2721, 2822, and 2823 are chemically related diamines in which the sulfur covering function is phosphate. These three compounds induced a similar clinical picture after intravenous administration. Prompt emesis, general depression, muscular weakness, paralysis of the nictitans, dyspnea, salivation and diarrhea are the dominant signs in the dog. These compounds are notably non-convulsant, and induce a clinical picture which is delayed and prolonged. The side effects are maximal at 2 to 3 hours and then gradually subside. In the previous annual report the specific ganglionic blocking activity of this series of compounds was reported. These three compounds in which the two nitrogen functions are separated by 3, 4 and 5 carbon chains differ strikingly from the compound WR 2578 in which the carbon chain length is 2. WR 2578 is not a ganglionic blocking agent, fails

to induce nictitans paralysis, is hypertensive, and somewhat better tolerated in the dog.

WR 2850B, an n-acetylated derivative of WR 1607, was administered orally in aqueous suspension, and induced vomiting which prevented evaluation of its ability to be absorbed.

The results of antiradiation studies in the dog and monkey are reported in the form of graphs # 1-6 indicating the total blood leukocyte counts and mortality of drug treated animals and their paired irradiated controls. Dotted lines indicate the leukocyte counts of drug treated animals and solid lines the leukocyte counts of the controls. Dotted and solid crosses indicate the days of death of treated animals and of controls respectively. Radiation exposures were conducted utilizing the 2 MEV Van der Graaff accelerator at the National Institutes of Health operated as an x-ray generator. Animals were irradiated in pairs, one treated animal and one untreated control per pair. The survival times of all control dogs irradiated at 450r under identical conditions over the past two years are presented in Graph #7. No dog of 93 irradiated has survived beyond 26 days. The mortality peaks sharply on days 12 to 14. The radiation dose rate was approximately 75r/min at a target to midline distance of 2 meters.

Compounds WR 2754 and 2691 were administered intravenously to dogs 15 minutes prior to lethal irradiation at 30 and 20 mg/kg respectively. No protective activity was observed for either of these related compounds. Convulsant activity prevented the administration of either of these compounds in greater doses.

Compounds WR 2578B and WR 2721 were also evaluated by intravenous administration. Excellent protective activity was demonstrated in both survival and leukocyte parameters. In both cases these agents were tested at doses inducing some drug-induced early mortality ("toxic death"). No radiation induced mortality was observed in any drug treated dog.

The results of antiradiation testing of two compounds in monkeys are also presented. The radiation LD₅₀/30 days under our conditions of irradiation is approximately 800r for the monkey, compared to an LD₅₀/30 days for the dog of 325r. Thus radiation protection experiments in the monkey were conducted at 1200r, a fully lethal dose. In monkey exposures, the target to midline distance was 1.5 meters and the dose rate approximately 150r/min.

(WR 2347, a polyfunctional alcohol derivative of mercaptoethylamine, having excellent protective activity in the mouse and dog at 300 mg/kg, was not effective in extending survival of monkeys exposed to this radiation dose.

WR 2529, a compound offering excellent protection in the mouse, also protected monkeys, significantly influencing the post-irradiation leukocyte response, and providing 30-day survival in 2 of 6 treated animals. This probably represents very significant protective activity in terms of radiation dose reduction, based on a comparison with results of WR 2347 and other previously reported monkey radiation results.

SUMMARY OF ANTIRADIATION DRUG TOLERANCE STUDIES IN THE DOG

July 1964 - June 1965


| WR # | Compound | Route | Dose mg/kg | # dead/# injected | Dominant Signs |
|--------|--|-----------------|---------------|----------------------|--|
| 1607-E | $\text{CH}_3(\text{CH}_2)_9\text{HNCH}_2\text{CH}_2\text{SSO}_3\text{H}$ | Oral | 25 | 0/1 | No evidence of absorption. No emesis. |
| | | in DMSO | 50 | 0/1 | |
| | | octanoic acid & | 75 | 0/1 | |
| | | cotton-seed oil | 150 | 0/1 | |
| 2691 |  $(\text{CH}_2)_4\text{HNCH}_2\text{CH}_2\text{SSO}_3\text{H}$ | I.V. | 15 | 0/1 | Emetic. Convulsant at 20 mg/kg. Hypertension, dyspnea, salivation, diarrhea. |
| | | | 20 | 0/2 | |
| | | | 25 | 1/1 | |
| | | Oral | 40 | | Emesis at 30 min. No evidence of absorption. |
| 2721 | $\text{H}_2\text{N}(\text{CH}_2)_3\text{HNCH}_2\text{CH}_2\text{SPO}_3\text{H}_2$ | I.V. | 100 | 0/3 | Emetic. Non-convulsant. Depression. Nictitans paralysis. Dyspnea, salivation, diarrhea. Signs delayed & prolonged. |
| | | | 200 | 1/3 | |
| | | | 300 | 0/1 | |
| 2822 | $\text{H}_2\text{N}(\text{CH}_2)_4\text{HNCH}_2\text{CH}_2\text{SPO}_3\text{H}_2$ | I.V. | 200 | 0/3 | Emetic. Non-convulsant. Depression. Nictitans paralysis. Dyspnea, salivation, diarrhea. Signs delayed & prolonged. |
| | | | 300 | 1/1 | |
| | | | 450 | 1/1 | |

Table 1

SUMMARY OF ANTIRADIATION DRUG TOLERANCE STUDIES IN THE DOG

July 1964 - June 1965

| WR # | Compound | Route | Dose mg/kg | # dead/# injected | Dominant Signs |
|--------|---|----------|---------------|----------------------|--|
| 2823 | $\text{H}_2\text{N}(\text{CH}_2)_5\text{HNCH}_2\text{CH}_2\text{SPO}_3\text{H}_2$ | I.V. | 150 | 0/2 | Emetic. Non-convulsant. Depression, nictitans paralysis. Dyspnea, salivation, diarrhea. Signs delayed & prolonged. |
| | | | 200 | 0/3 | |
| | | | 300 | 1/1 | |
| 2850-B | $\text{CH}_3(\text{CH}_2)_9\text{N}(\text{CH}_2)_2\text{SSO}_3\text{Na}$ $\begin{array}{c} \text{C=O} \\ \\ \text{CH}_3 \end{array}$ | Oral | 200 | 0/1 | Vomiting in 20-35 min. No evidence of absorption. |
| | | Aq.Susp. | 400 | 0/1 | |
| | | | 800 | 0/1 | |

Table 2

Graph 1

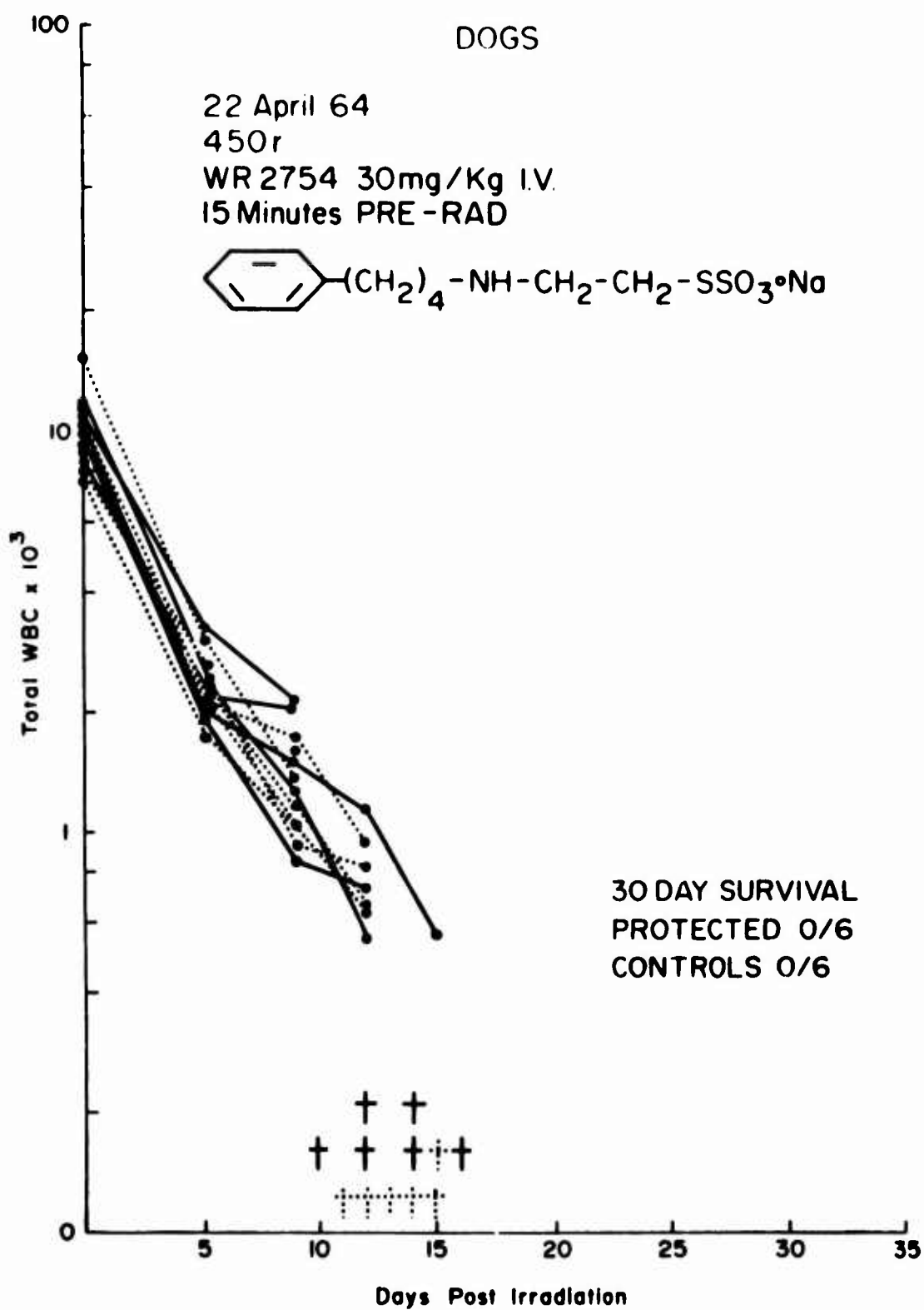
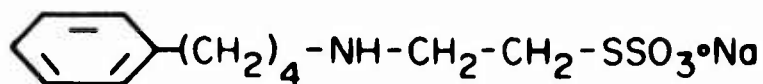
DOGS

22 April 64

450r

WR 2754 30mg/Kg I.V.

15 Minutes PRE-RAD



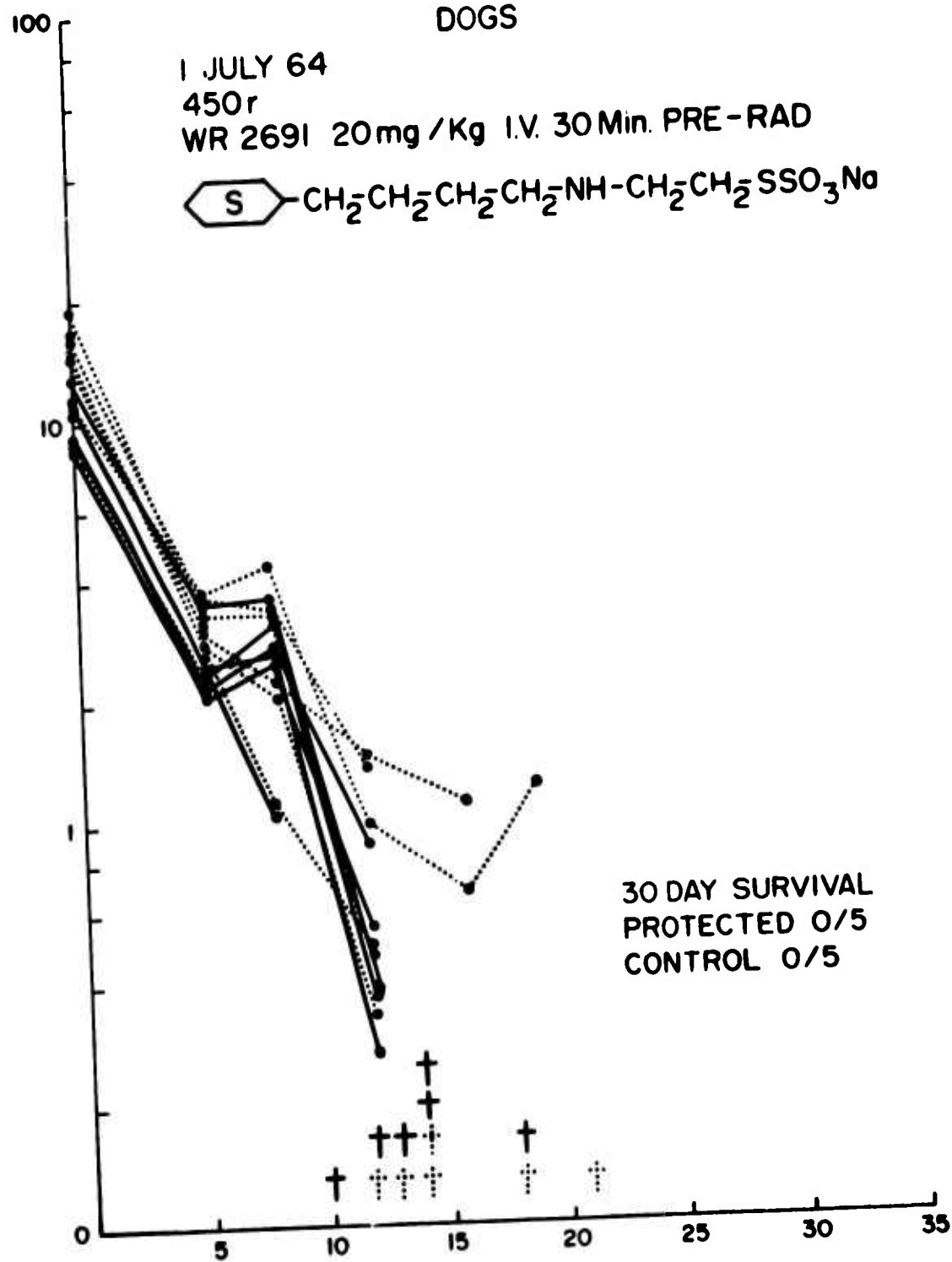
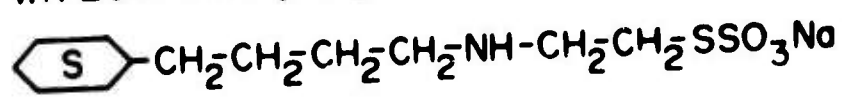
Graph 2

DOGS

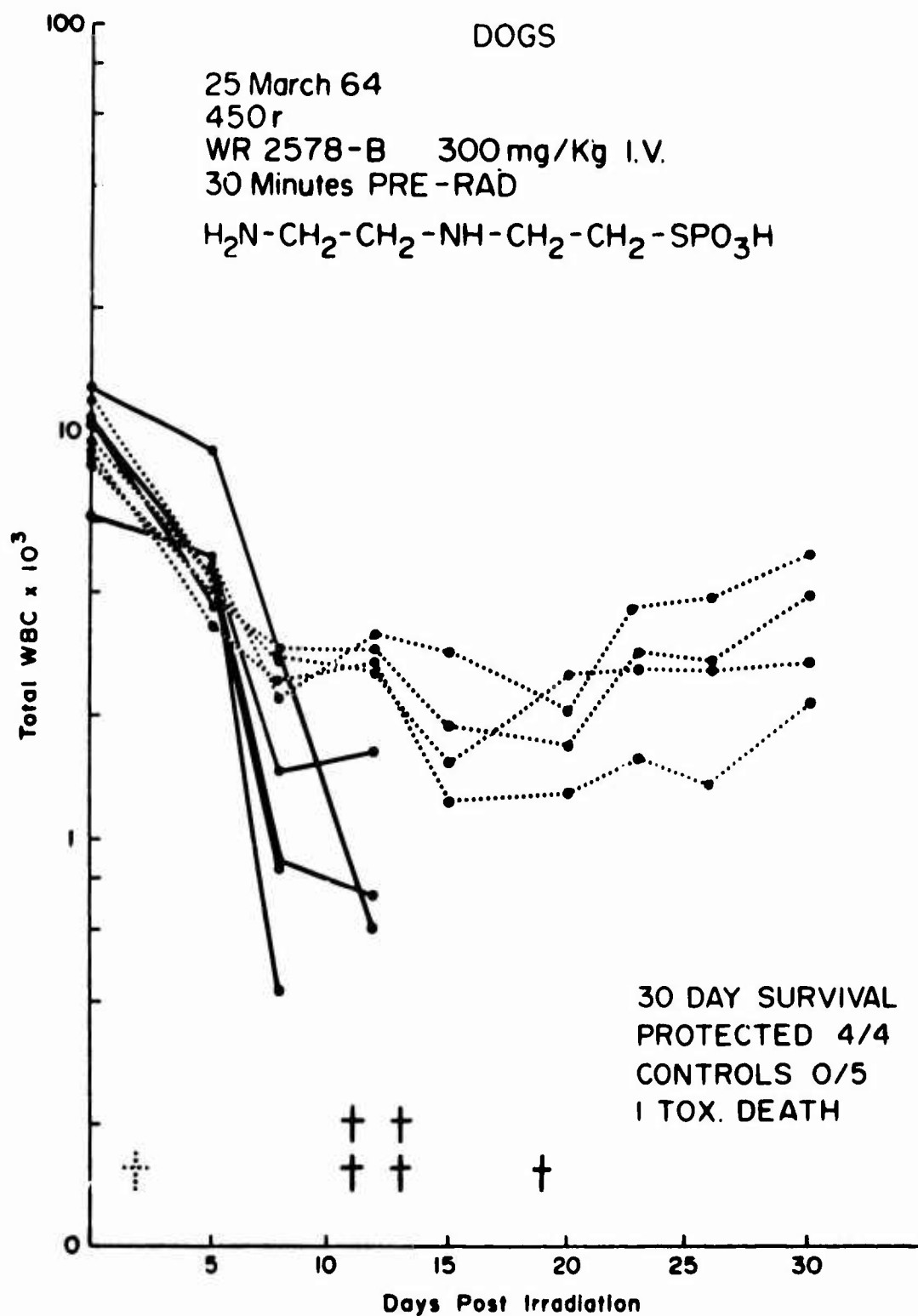
1 JULY 64

450r

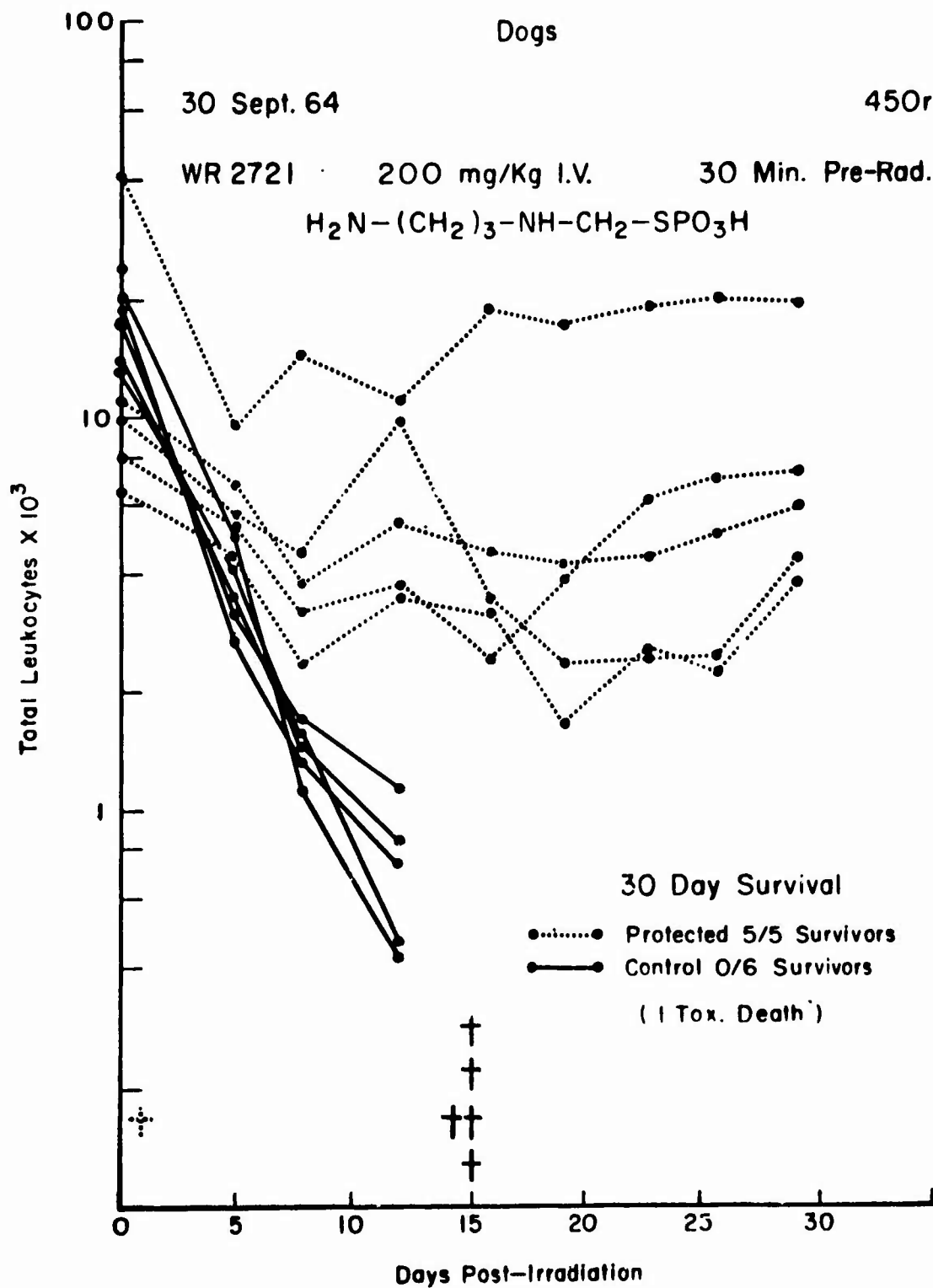
WR 2691 20mg / Kg I.V. 30 Min. PRE-RAD



Graph 3



Graph 4



Graph 5

MONKEYS

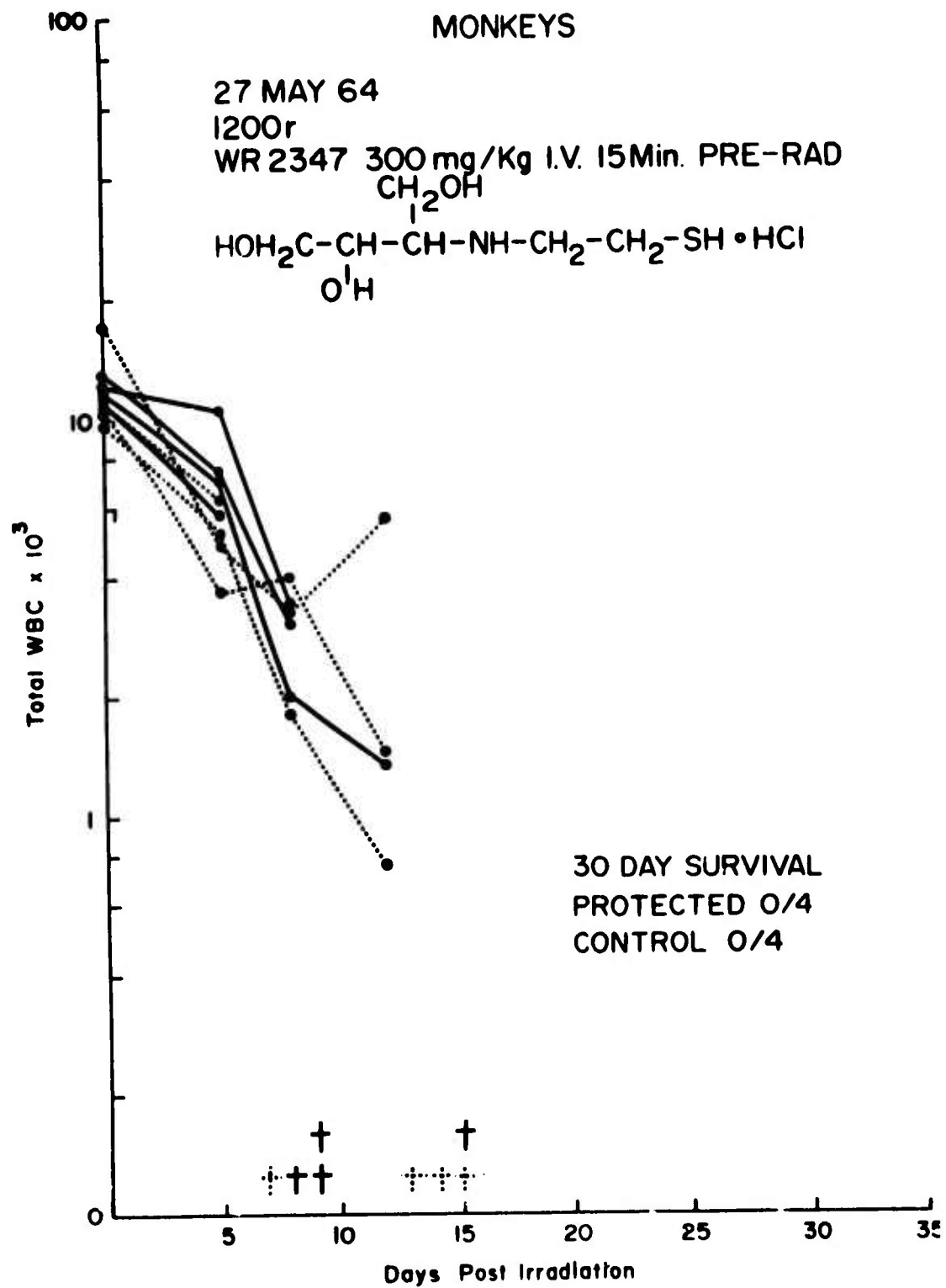
27 MAY 64

1200r

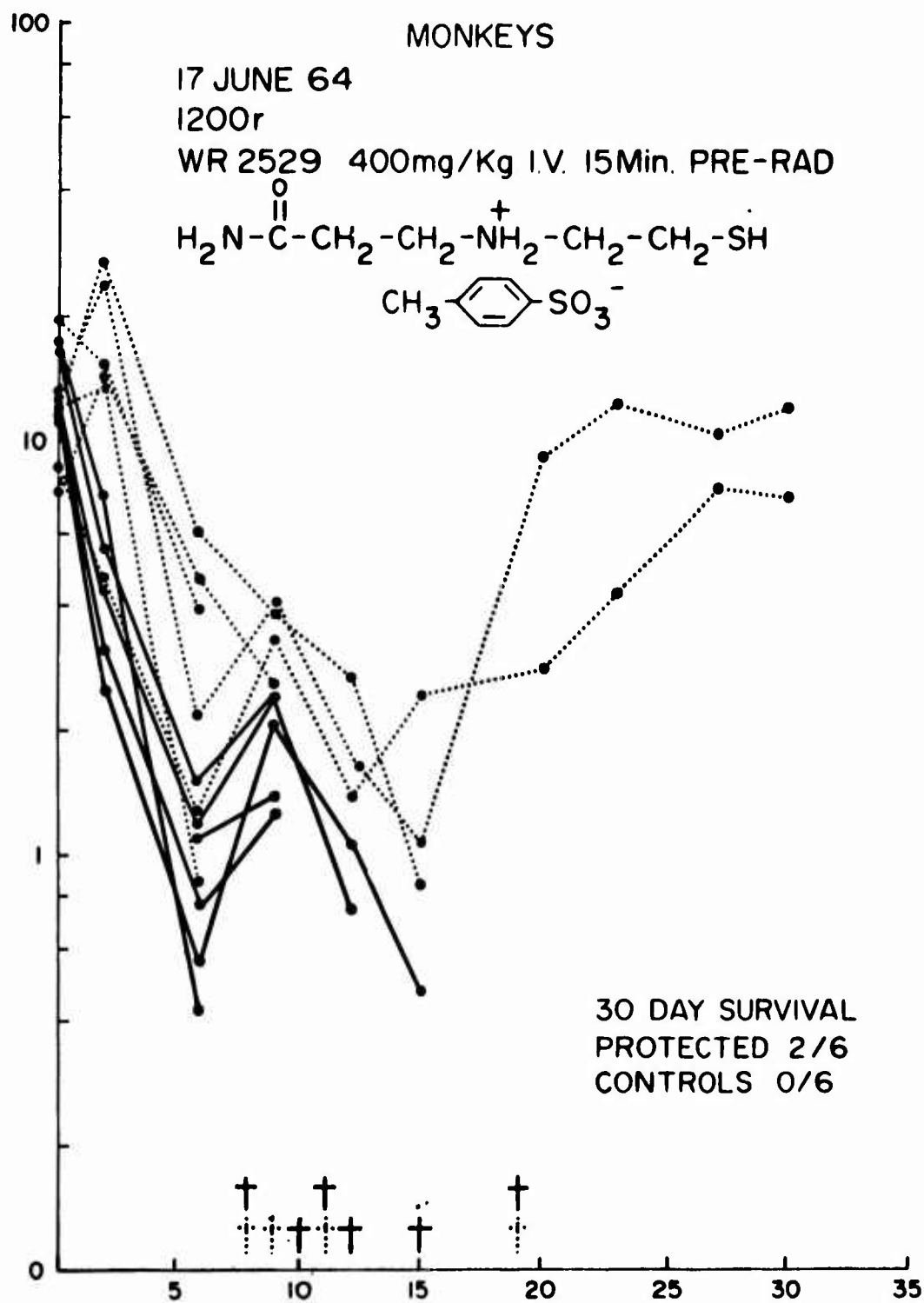
WR 2347 300 mg/Kg I.V. 15 Min. PRE-RAD

CH₂OH

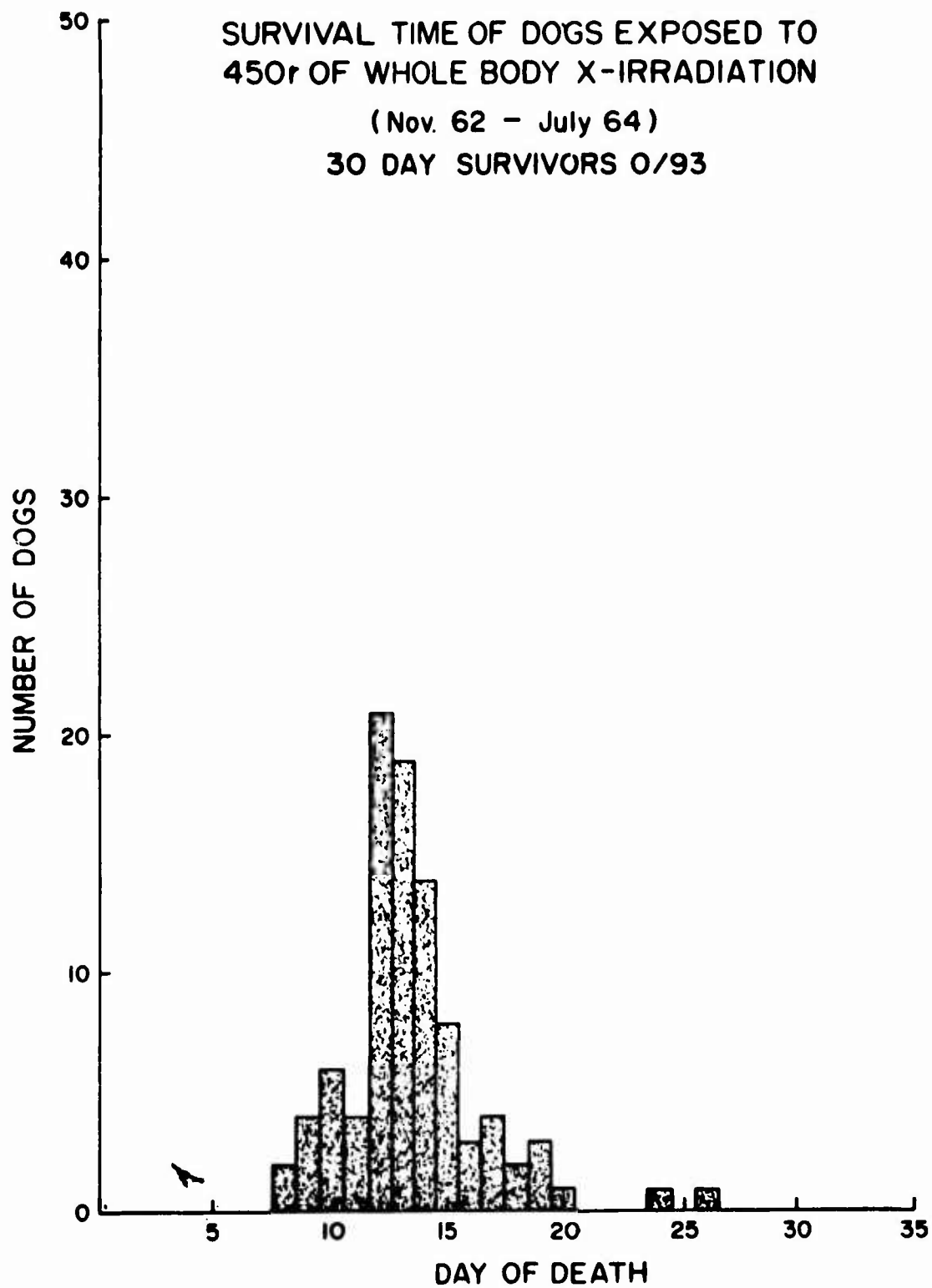
HOH₂C-CH-CH-NH-CH₂-CH₂-SH • HCl
O¹H



Graph 6



Graph 7



D. Evaluation of Emetic Activity in Large Animals

Studies have been initiated to evaluate the emetic activity of candidate antiradiation agents in dogs and in cats. An indwelling Feldberg canula modified and evaluated by Dr. Herbert Borison of the Dartmouth University Medical School has been utilized for maintaining chronic access to the lateral ventricles of the cerebrum. The dogs and cats utilized in the preliminary studies reported here were furnished by Dr. Borison. Chemical agents administered intracranially in cats were dissolved in 0.25 ml of sterile saline. Dr. Borison has demonstrated that this volume of material is immediately distributed through the lateral ventricle, third ventricle and fourth ventricle upon injection into the canula. A volume of 0.5 ml was utilized in the dogs. Dead space within the canula is less than 10 microliters.

An important advantage of the intracranial route of administration is the ability to elicit an emetic response utilizing only small quantities of test material. This will permit the evaluation of central emetic activity of critical agents available in too small quantity for intravenous administration. Information regarding central versus peripheral site of action might also be obtained. It is hoped that some information regarding the mechanism of action of the emetic activity of radioprotective compounds might also be gained through the use of animals with experimentally induced lesions in the emetic centers of the brain.

The emetic response of dogs and cats to intravenously administered 2-mercaptoethylamine hydrochloride (MEA) was evaluated. A marked species difference was observed. MEA was administered in isotonic solution at a pH of 7.2. An emetic response was elicited in all dogs in 3 to 14 minutes following rapid intravenous administration of 25 mg/kg or greater. No emetic response was observed in the cat at doses less than 70 mg/kg. Central nervous system stimulation with hyperactivity and tonic-clonic convulsions was observed in the cat at 60 mg/kg. Previous work in this laboratory indicates that the convulsant threshold for MEA in the dog is in excess of 75 mg/kg (Annual Report 1964). While the convulsant activity of MEA is quantitatively not greatly different between the dog and cat, the dog seems particularly sensitive to the emetic effect.

Four related thiophosphates of MEA were evaluated by intravenous administration in cats (WR 2578, WR 2721, WR 2822, and WR 2823). None of these compounds exhibited convulsant activity. WR 2578 elicited an emetic response only at doses in excess of 200 mg/kg. WR 2721, 2822 and 2823 were emetic at doses of 50 mg/kg or less. Emesis usually occurred between 10 and 20 minutes, although occasionally delayed for up to one hour. These four compounds are also emetic in the dog, but have not been

evaluated at doses of less than 250 mg/kg (Annual Report 1964). WR 2347, a polyfunctional alcohol with radioprotective and emetic activity in the dog was also administered intravenously to cats. Both emetic and convulsant activity were observed at doses of 240-300 mg/kg. In the dog, emesis and occasionally convulsions are observed at this dose level, but lower doses have not yet been evaluated.

Emetic activity of some of these compounds has been observed following intracranial administration. In the cat, two polyfunctional alcohols, WR 2347 and WR 1616, were convulsant intracranially at 5 mg/kg without emetic activity.

WR 2822 and 2823 were administered intracranially in both dogs and cats. Although the number of experiments so far completed is insufficient to permit definite conclusions to be drawn, it appears that the dog and cat are not greatly different in their emetic response to these agents. Emesis after intracranial administration occurs at about 1/50 to 1/100 of the intravenous emetic dose.

Table 1

COMPARISON OF EMETIC RESPONSE OF DOG AND CAT TO MEA
(IV Administration)

| <u>Mg/Kg</u> | <u>Dog</u> | <u>Cat</u> |
|--------------|--------------------|------------------------------|
| 25 | 3/3 (11,14,14 min) | |
| 32 | 2/2 (11,14 min) | |
| 40 | 1/1 (3 min) | 0/1 |
| 50 | 1/1 (8 min) | 0/1 |
| 60 | | 0/1 (Hyperactivity) |
| 70 | | 1/2 (1 min) (Convulsions) |

CAT INTRACRANIAL ADMINISTRATION

| <u>Compound #</u> | <u>Drug Dose (mg/kg)</u> | <u>Animals Vomiting</u> |
|-------------------|--------------------------|-------------------------|
| WR 347 (MEA) | 0.50 | 0/1 |
| | 0.75 | 0/1 |
| WR 1616 | 5.0 | 0/1 Convulsions. Death. |
| | 7.0 | 0/1 Convulsions. Death. |
| WR 2347 | 2.5 | 0/1 |
| | 5.0 | 0/1 Convulsions. |
| WR 2822 | 1.00 | 3/3 (10,13,16 min) |
| WR 2823 | 0.25 | 0/1 |
| | 0.38 | 0/2 |
| | 0.50 | 3/4 (8,8,16 min) |
| | 0.75 | 3/4 (5,13,23 min) |
| | 1.00 | 3/5 (9,12,60+ min) |

DOG INTRACRANIAL ADMINISTRATION

| | | |
|--------------|------|------------------|
| WR 347 (MEA) | 0.25 | 0/2 |
| WR 2822 | 0.50 | 1/1 (4 min) |
| | 1.00 | 1/1 (9 min) |
| WR 2823 | 0.25 | 1/2 (35 min) |
| | 0.50 | 2/2 (14,60+ min) |

Table 2

Table 3

Emetic Responses of Cats - Intravenous Administration

| <u>Compound #</u> | <u>Drug Dose (mg/kg)</u> | <u>Animals Vomiting</u> |
|-------------------|--------------------------|---------------------------------|
| WR 2578-B (n=2) | 25 | 0/1 |
| | 50 | 0/1 |
| | 100 | 0/1 |
| | 150 | 0/2 |
| | 200 | 3/3 (11,25,34 min) |
| WR 2721 (n=3) | 25 | 0/1 |
| | 38 | 1/2 (20 min) |
| | 50 | 4/5 (15,18,18,39 min) |
| | 75 | 3/3 (12,17,23 min) |
| WR 2822 (n=4) | 12 | 0/1 |
| | 18 | 0/2 |
| | 25 | 2/3 (10,11 min) |
| | 38 | 1/1 (42 min) |
| | 50 | 7/7 (4,8,9,11,11, 11,14 min) |
| | 100 | 1/1 (8 min) |
| | 200 | 1/1 (8 min) |
| WR 2823 (n=5) | 12 | 0/1 |
| | 25 | 1/2 (18 min) |
| | 38 | 2/2 (14,16 min) |

Table 4

CAT INTRAVENOUS ADMINISTRATION

| <u>Compound #</u> | <u>Drug Dose (mg/kg)</u> | <u>Animals Vomiting</u> |
|--|--------------------------|---|
| WR 2347 | 200 | 0/1 |
| | 240 | 1/1 (32 min) |
| $\text{HOCH}_2\text{CHOH}-\overset{\text{CH}_2\text{OH}}{\underset{\text{HCL}}{\text{C}}}-\text{HNCH}_2\text{CH}_2\text{SH}$ | 300 | 3/5 { (14, 24, 28 min) (2/5 convulsions) |
| | 400 | 0/1 (convulsant) |

E. Evaluation of Mathematical Program

Dr. Alfred Lehman continues to work in Advanced Algebra. His studies have centered around matroids and the encoding of planar graphs. During the past year he participated in the seminar at Rand' involving matroid theory with specific application of the Shannon Switching Game. His studies of chemical structures as graphs have resulted in an evaluation of their properties from the point of view of fundamental graph theory. This insight is essential to proper handling of the structures in large computer systems. Two papers on this subject were presented and a long article is in process.

| RESEARCH AND TECHNOLOGY RESUME | | | 1. | 2. GOVT ACCESSION | 3. AGENCY ACCESSION | REPORT CONTROL SYMBOL |
|--|--------------------|-----------------------|---|-----------------------|----------------------------|-----------------------|
| | | | | | DA OA6472 | CSCRD-103 |
| 4. DATE OF RESUME | 5. KIND OF RESUME | 6. SECURITY | 7. REGRADING | 8. RELEASE LIMITATION | 9. LEVEL OF RESUME | |
| 01 07 65 | A. NEW | U RPT U WRK | NA | QR | A. WORK UNIT | |
| 10a. CURRENT NUMBER/CODE | | | 10b. PRIOR NUMBER CODE | | | |
| 62156011 3A025601A824 01 056 | | | None | | | |
| 11. TITLE | | | | | | |
| (U) Mechanisms (09) | | | | | | |
| 12. SCIENTIFIC OR TECH. AREA | | | 13. START DATE | 14. CRIT. COMPL. DATE | 15. FUNDING AGENCY | |
| 014100 Radiobiology; 011500 Nuclear Weapons Effects | | | 09 63 | NA | OTHER DA | |
| 16. PROCURE. METHOD | 17. CONTRACT/GRANT | | 18. RESOURCES EST. | | 19. PROFESSIONAL MAN-YEARS | |
| C. IN-HOUSE | a. NUMBER | | PRIOR FY | | 317 | |
| | c. TYPE NA | | CURRENT FY | | 350 | |
| 19. GOV'T LAB/INSTALLATION/ACTIVITY | | | 20. PERFORMING ORGANIZATION | | | |
| NAME Headquarters | | | NAME Walter Reed Army Institute of Research | | | |
| ADDRESS U.S. Army Medical Res & Dev Command | | | ADDRESS Washington, D. C. 20012 | | | |
| RESP. INDIV Goldstein, Col. J.D. | | | INVESTIGATORS Woodward, Lt. Col. K.T. | | | |
| TEL 202-0X 65957 | | | PRINCIPAL Hightower, Lt. Col. D. | | | |
| | | | ASSOCIATE Angel, Maj. C. R. | | | |
| | | | TEL 202-576-2211 TYPE DA | | | |
| 21. TECHNOLOGY UTILIZATION | | | 22. COORDINATION | | | |
| Civil Defense; Radiobiology; Radio-Radiation Chemistry | | | NA | | | |
| 23. KEYWORDS | | | | | | |
| Cytogenetics; radiation injury; radiobiology | | | | | | |
| 24. (U) Tech Objective - The quantitative definition of the mechanisms of ionizing radiation injury in molecular and living systems. | | | | | | |
| <p>(U) Approach - Molecular, cellular, and mammalian species are utilized to evaluate radiation injury and repair using various chemical, physical and biological end points of response. Paramount are cytogenetic, histopathologic, and radioautographic studies, the biological effectiveness of certain aminoethiols in reducing radiation injury, and the influence of radiation injury on biochemical systems and trace elements.</p> <p>(U) Progress (Jul 64 - Jun 65) - Electron spin resonance spectrometry has been utilized to measure quantitatively and qualitatively free radicals in normal and irradiated systems and the influence of radiomodifiers. Chromosome changes in primate lymphocyte cultures have been studied as an index of residual injury in mammals. Effectiveness of aminoethiols in reducing radiation lethality in rodents has been studied as a function of type of radiant energy (X, gamma and neutron). Split radiation dosage has been used to assess residual injury.</p> <p>For technical reports, see Walter Reed Army Institute of Research Annual Progress Report, 1 July 1964 - 30 June 1965.</p> | | | | | | |
| 27. COMMUNICATIONS SECURITY | | 28. | 29. OSD CODE | | 30. BUDGET CODE | |
| <input type="checkbox"/> COMSEC OR COMSEC RELATED <input checked="" type="checkbox"/> NOT RELATED | | | AR | | 1 | |
| 31. MISSION OBJECTIVE | | | 32. PARTICIPATION | | | |
| 1212b(9) | | | NA | | | |
| 33. REQUESTING AGENCY | | 34. SPECIAL EQUIPMENT | | | | |
| | | | | | | |
| 35. EST. FUNDS (In thousands) | | 36. | | | | |
| | | | | | | |

DD FORM 1498
1 AUG 64

(Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

Project 3A025601A824, IONIZING RADIATION INJURY, PREVENTION AND TREATMENT

Task 01, Ionizing Radiation Injury, Prevention and Treatment

Work Unit 056, Mechanisms

Investigators.

Principal: Lt Col Kent T. Woodward, MC

Associate: Major Charles R. Angel, MSC; Billy G. Bass, B.S.; Ann R. Berman, B.S.; John Davis, B.S.; Minnie H. Davis, B.S.; Milton H. Feldman, Ph.D.; Capt Fletcher Hahn, VC; Lt Col Dan Hightower, VC; Major Merrill Johnson, MC; Adolph T. Krebs, Ph.D.; Mary M. McLaughlin, M.S.; Major Harold M. Swartz, MC, Depts Biophysics, Isotope Metabolism and Radiation Biology; Marie M. Grennan, M.S., Dept of Medicinal Chemistry.

Description.

The objective of this work unit is to define mechanisms by which ionizing radiations affect living systems and to devise methods of prevention, modification, and treatment. Multidisciplined investigations include

1. Free Radical Measurements

Utilizing Electron Spin Resonance (ESR) Spectrometry radiation induced free radicals were studied in a variety of materials. Particular emphasis was placed on defining suitable biological systems in which resonance patterns and biological response to treatment could be measured in the presence of radiation modifiers.

2. Submanmalian Species Response

The effect of ionizing radiation on volitional activities of several strains of radioresistant insects was studied to measure comparative injury and recovery patterns.

3. Mammalian Studies

The definition of changes in functional integrity and chemical consistency after exposure to ionizing radiation have been explored.

a. The influence of selected aminothiols on dose mortality relationships after neutron irradiation.

b. The effect of multiple radiation exposures on mortality with and without preprotective treatment.

c. The development of cell culture techniques for the study of cytogenetic effects of irradiation.

d. The development of neutron radioactivation analytic techniques for the study of trace elements (free and organically bound) with particular reference to radiation injury.

Progress.

1. Free Radical Studies.

Progress in ESR spectrometry has been divided into the following:

a. Instrumental and equipment considerations

b. Naturally occurring resonances

c. Radiation induced ESR resonances

(1) Biological considerations

(2) Results in irradiated biological material

a. Instrumental Considerations. ESR spectroscopy is in a state of rapid development and particular progress on two aspects of instrumentation has been made. (1) A device to permit accurate and easy variation of sample temperature between -196°C . and $+300^{\circ}\text{C}$. has been obtained and standardized. This will permit additional kinetic studies and will aid in the resolution of different radical species. (2) ESR spectra are first derivatives necessitating double integration. Mathematical and electronic requirements have been defined and suitable instrumentation to perform these operations (as well as signal digitalization and signal enhancement by repetitive scanning) is now commercially available.

b. Naturally Occurring Resonances. The ESR spectrometer will detect any unpaired electron species including paramagnetic elements, conduction band electrons, naturally occurring free radicals and, as well, radiation induced free radicals. Consequently work has been done on unirradiated materials to determine "natural" ESR spectra before irradiation, as well as their suitability for ESR observation in terms of excess nonspecific absorption (due to too much material with high dielectric constants). Such absorption renders many biological materials unsuitable for ESR observations.

A large variety of insects (ants, mosquitoes, beetles, flies) were examined and found suitable for ESR investigations. In ants natural signals included absorptions due to (1) manganous (Mn^{++}) ion, (2) melanin, (3) a nonmelanin pigment. Studies indicate that biliverdin and bilirubin or a closely related compound are involved in the non-melanin pigment signal. Other insects also showed "natural" ESR signals,

including complex signals in insects that secrete quinoid compounds in large quantities.

Snails also proved suitable for ESR investigation and showed a naturally occurring signal at the free radical area ("g=2 area") as well as an Mn^{++} signal.

Mouse and kangaroo rat tails were found to be "dry" enough for ESR investigation. A special sling was constructed to permit insertion of the tails into the ESR spectrometer. A weak g=2 resonance was found.

A technique to observe green plants under physiological conditions was developed and the natural background signal of winter rye seedlings was determined to be devoid of a g=2 signal. Exposure of rye seedlings to visible light produced a g=2 signal if air or oxygen was present, but not in 100% N_2 . High concentrations of CO_2 decreased the intensity of the light induced signal.

Bacteria were examined at room temperature (in a special holder to minimize nonspecific absorption) and frozen in both aqueous and lyophilized states and found to have a g=2 signal in all cases. The signal in lyophilized bacteria occurred only on exposure to oxygen and was decreased by substances that are also protective against radiation. Repeated freezing of hydrated cells also increased the g=2 signal. These systems are to be investigated with other active and inactive radioprotective drugs added to determine if a common toxic pathway exists (see below on irradiated bacteria).

Normal dog tissue was examined and found to have readily detectable g=2 signals when frozen to $-196^\circ C$. A controversy in the literature about natural tissue signals was resolved by a demonstration that spectra change markedly depending on microwave power.

c. Radiation Induced ESR Signals.

(1) Biological considerations. Biological systems have been investigated in which viability and growth, as well as ESR patterns, could be measured on the same specimen in the presence of radiation modifiers. This imposes very severe limitations. Biological systems are being sought that will not cause excess nonspecific microwave absorption (due principally to liquid water) and yet show radiation induced ESR signals and maintain viability. To observe very short-lived radicals the ability to withstand low temperatures ($-196^\circ C$.) must also be present. "Classical" results relating ESR parameters and radiation modifiers was repeated adding a test of viability and pre-irradiation treatments alone (quick freezing of deuterated yeast) reduced viability to 2% or less. Alternate methods were then attempted, including slow freezing of yeast (50% - 70% survival) and quick freezing of *E. coli*

B/r (approximately 100% survival). The latter organism was chosen for further study because of higher survival, easier growing conditions, lack of budding, and the vast amount of data available on *E. coli*.

(2) ESR results on irradiated biological systems.

Bacteria. A general correlation between the reduction of ESR signal in irradiated frozen *E. coli* and survival, in the presence of mercaptoethylamine (MEA), was found. In particular as the concentration of MEA was changed the area of most rapid ESR modification corresponded with the area of most rapid viability change. The non-protective disulphide of MEA (cystamine) did not produce ESR changes similar to those seen with MEA. Other analogues are being screened with this system.

Insects. Two different species of ants after 82,000 rads (Co^{60} gamma) showed similar ESR signals with at least two components to the decay curve (in the order of minutes and of hours). An irradiated two-spot lady bug showed a nondecaying ESR signal similar to irradiated chitin.

Snails. All species showed a stable radiation-induced ESR signal and a dose response curve was obtained. Separation of the body from the shell showed most of the signal to be in the calcium carbonate shell.

Mammals. Following 5,000 rads to the tail of the kangaroo rat an ESR signal was detected which slowly decayed over several hours.

Human blood, irradiated *in vitro* at 20° C. showed an ESR signal, persistent for at least hours, that is proportional to dose over a wide range. This is the first reported occurrence of a detectable radiation induced ESR signal in a biological system with no large dry areas. The signal seen in blood shows definite hyperfine structure.

Bones and teeth of rats and monkeys irradiated *in vivo* have been shown to have persistent ESR signals proportional to dose with a three component decay curve (seconds, minutes, and hours to days). The resonances apparently lie primarily in the calcium phosphate matrix of the bone.

Frozen dog tissues (-196° C.) demonstrate a linear dose response curve to gamma irradiation. The resonances persist to some extent to -77° C.

Several systems have been investigated that show promise of yielding combined ESR patterns and biological responsiveness after irradiation.

Relatively stable radiation induced electron spin resonances have been found in some mammalian tissues. These may affect current radiobiological theory (which does not take into account such long-lived energy sources) and may also offer potential *in vivo* dosimeters.

2. Submammalian Species Response.

Radiation induced changes in the tunneling activity of the California red harvester ant (*Pogonomyrmex californicus*) has been used as a comparative response in the volitional activity of a highly radio-resistant organism.

Ants were exposed to graded doses of Co^{60} gamma rays at a dose rate of 1300 r/minute. Thereafter periodic observations were made of tunneling activity and work performance, length of survival, and mortality. Graded sigmoidal response curves have been obtained for these three functions for comparison with rodent, canine, and primate studies.

3. Mammalian Studies.

a. Effectiveness of Radiation Modifiers on Reactor Radiation Exposure Responses. The radiation lethality and survival time responses after whole body irradiation may be modified by

- (1) Strain and age
- (2) Type of radiation
- (3) Total dose and dose rate
- (4) Single or fractionated exposures
- (5) Trauma or infection
- (6) Anoxia and certain preradiation stresses
- (7) Chemoprophylactic agents
- (8) Bone marrow implantation

From the synthesis and testing of mercaptoethylamine analogs for radioprotective effect against x- or gamma-irradiation, a number of active agents have become available for manipulating radiation lesions and possibly reparative processes. Measurement of the comparative effectiveness of these compounds in reducing mortality from several types of radiation is of fundamental importance.

Since the spatial distribution of ionizing events in tissue is dependent on type and quality of the incident radiation, the consequent biological expression of effect also varies with type and quality of radiation used. In general biological effectiveness increases with higher linear transfer of energy per unit path in tissue and a maximum effectiveness would be produced by those particles producing precisely the right number of ionizations in the target volume. Radiations producing more ionizations than necessary to disrupt the target volume would be less effective per unit of dose as part of the radiant energy would be supersaturating or wasted. For various ionizing radiations

available at this Institute the fundamental differences in absorption characteristics are

| Type of Radiation | Interaction with Tissue | Range in Tissue | Linear Energy Transfer in Tissue | Oxygen Dependency for Biological Effect |
|---------------------|--------------------------------------|---------------------|----------------------------------|---|
| x and γ rays | Electrons | 30-6,000 μ^1 | 0.3-3 Kev/ μ | Yes |
| Fission Neutrons | Recoil Protons | $\sim 20 \mu^2$ | 45 | Minimum to No Effect |
| Thermal Neutrons | 34% n, p (N^{14}, C^{14}) | $\sim 10 \mu^2$ | -65 Kev/ μ | No |
| | 20% n, γ (H^1, H^2) | $\sim 6,000 \mu^2$ | -0.3 Kev/ μ | Yes |
| | 33% n, γ (C^{12}, C^{13}) | $\sim 10,000 \mu^2$ | -0.2 Kev/ μ | Yes |

¹Approximate range of incident radiation

²Approximate range of secondary particle or photon

A series of experiments have been conducted in two strains of mice to assess the effectiveness of selected chemoprophylactic agents (singly or in combination) in modifying length of survival and percent mortality after exposure to reactor radiations (LET ~ 45 Kev/micron).

Radiation exposure factors are

(1) Co^{60} gamma ray exposure

- (a) Source strength - 900 curies
- (b) Dose rate - 85 r/minute
- (c) Exposure - 20 mice per group in rotating leucite chamber

(2) Reactor radiation exposure

- (a) Fast neutron flux - 3.6×10^4 n/cm²/watt sec.
- (b) Thermal neutron flux - 3.5×10^4 n/cm² watt sec.
- (c) Cadmium ratio - 4:1
- (d) Neutron to gamma ratio - 5.5:1
- (e) Dose rate at power - 0.097 rad/watt min
(~ 85 rad/min at 850 watts)
- (f) Transit dose to power - 46 rads

Neutron flux spectra are available for the Walter Reed Reactor as compared to other neutron sources used in research. Groups of 12 mice were exposed in graphite tubes (2 mice per tube) using a 12 x 12 inch exposure void in the south column of the reactor.

Thereafter animals were housed together and observed daily for mortality. The results of these experiments are in large part due to the experience and excellent technical assistance rendered by personnel in the Department of Medicinal Chemistry. Approximate dose reduction factors for cobalt-60 gamma ray and reactor radiation exposures are shown in tabular form. Additional information on drug dosage and toxicity, duration of effect, etc. are reported under Work Unit 055, Chemoprophylaxis.

Three drugs (MEG - 100 mpk and GSH - 1000 mpk in combination; and serotonin - 100 mpk alone) were selected for baseline studies of radioprotective effect. Additional selections were made on the basis of radioprotective activity against X or gamma ray exposures. Approximately 15 to 20 minutes transpired between administration of compound(s) and the onset of radiation exposure. Saline administered controls were included in and during each exposure with radioprotected animals.

| Experimental Group | Walter Reed Drug Accession Number | Approximate Dose Reduction Factor Co ⁶⁰ γ Radiation | Approximate Dose Reduction Factor Reactor Radiation |
|--------------------|-----------------------------------|--|---|
| A | MEG 100 mpk GSH 1000 mpk | 1.6 | 1.3 |
| B | Serotonin 100 mpk | 1.8* | 1.1 |
| D | 638 | 1.6 | 1.3 - 1.4 |
| J | 1607 | 1.4 | 1.1 |
| C | 1607, 302 | 1.6 | 1.0 |
| E | 1607, 302, 638 | 2.2 | 1.3 |
| I | 2578 | 1.8 | 1.0 |
| K | 2721 | > 2.2 | 1.2 |
| H | 2822 | 2.1 | 1.1 |
| G | 2822, 539, 347 | 2.3 | 1.2 |
| F | 347, 539 | 2.1 | 1.1 |
| L | 2529 | 1.7 | 1.2 |

*Literature value.

The DRF's obtained for reactor radiations are all lower than for Co^{60} gamma radiation. Drug #638 alone or in combination demonstrated activity comparable to MEG-GSA using reactor radiations.

b. Effectiveness of Radiation Modifiers on Multiple Radiation Exposures to Co^{60} and Reactor Radiations. Groups of C-57 female mice (preprotected with MEG-GSH and saline controls) were exposed to graded doses of Co^{60} or reactor radiations at 30-day intervals and observed for mortality. Dose reduction factors were estimated for each exposure experience.

Although the estimated median lethal dose decreases for each subsequent exposure the dose reduction factor for MEG-GSH protection remains essentially constant as shown in the following tabulation.

Co^{60} Radiation Exposure

| <u>Exposure</u> | <u>1</u> | <u>2</u> | <u>3</u> |
|------------------------|----------|----------|----------|
| Median Lethal Dose (r) | | | |
| Control | 785 | 740 | 650 |
| Preprotection | 1280 | 1125 | 1030 |
| Approximate DRF | 1.63 | 1.52 | 1.58 |

Reactor Radiation Exposure

| <u>Exposure</u> | <u>1</u> | <u>2</u> |
|--------------------------|----------|----------|
| Median Lethal Dose (rad) | | |
| Control | 315 | 257 |
| Preprotection | 435 | 335 |
| Approximate DRF | 1.38 | 1.30 |

c. Cytogenetics in Radiation Biology. Ionizing radiation is well known for the induction of chromosome abnormalities in somatic and genetic tissues. Considerable effort has been directed to define the conditions required for bone marrow and peripheral blood cultures in laboratory animals, particularly the monkey. A modified Moorhead culture technique has been utilized.

Factors which have been investigated include anesthesia (when required for sampling), composition of media, phytohemagglutinin activity, and the concentration of lymphocytes and granulocytes in the inoculum.

Sernyl anesthesia (Parke Davis) is rapid in onset, of short duration, and has no apparent antimitotic effect on subsequent blood culture. The culture media of Humalson was most consistently successful, although 80% TC 199 and 20% fetal calf serum was only slightly less successful. Phytohemagglutinin activity was quite variable and lacks standardization. The concentration of lymphocytes is an important variable and the optimum inoculation varied from 50,000 to 75,000 lymphocytes and there was a definite impression that elevated granulocyte counts interfered with optimum lymphocyte mitosis. Present culture techniques show 80% blast formation with mitotic figures suitable for karyotypic analysis in about 66% of the cultures set.

Marrow or peripheral blood cultures show little application in the immediate post-irradiation period for various technical limitations imposed by lymphocytopenia, chromosome stickiness, etc. Major emphasis resides with assessing residual chromosome damage after reparative hematopoietic processes have been established.

d. True Elements in Biology and Medicine. With rapid advances in techniques for neutron radioactivation of trace elements in complex matrices and the availability of the Walter Reed Research Reactor considerable effort has been directed toward the analysis of elements of known biological importance including manganese, copper, zinc, fluorine, and serum protein bound iodine. Single elemental analyses are available for small numbers of samples. Alternative methods of analysis are under development including semi-automated sequential techniques to provide capabilities for multiple elemental analysis in large numbers of samples with minimal technical assistance and reduced cost per sample element.

To augment present Walter Reed capabilities a contract with General Atomics has been let. Objectives are (1) development of sequential analytic techniques applicable to the Walter Reed Reactor and (2) a temporal 1-year survey of a normal healthy adult population residing in the Washington, D.C. area and a randomly selected population with extremes in adult age. Elements under study are copper, manganese, zinc, selenium, strontium, iodine, vanadium, cobalt and molybdenum.

Summary and Conclusions.

Within this work unit investigations are directed to the study of ionizing radiation injury and repair utilizing multidisciplinary approaches including (1) ESR spectrometry of free radical production, (2) radiation modifiers using radiations with various LET, (3) hematology and cytogenetics and (4) radiation pathology.

Publications.

Swartz, Harold M.: Long-lived Radiation Induced Electron Spin Resonance Signals *in vivo*. *Radiation Research* 22:242, May 1964.

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